Substance Name:	**1,6,7,8,9,14,15,16,17,17,18,18-Dodecachloropentacyclo[12.2.1.1^{6,9}.0^{2,13}.0^{5,10}]octadeca-7,15-diene ("Dechlorane Plus"TM) [covering any of its individual anti- and syn-isomers or any combination thereof]**
EC Numbers:	**236-948-9; -; -**
CAS Numbers:	**13560-89-9; 135821-74-8; 135821-03-3**

MEMBER STATE COMMITTEE

SUPPORT DOCUMENT

FOR IDENTIFICATION OF

1,6,7,8,9,14,15,16,17,17,18,18-DODECACHLOROPENTACYCLO[12.2.1.1^{6,9}.0^{2,13}.0^{5,10}]OCTADECA-7,15-DIENE ("DECHLORANE PLUS"TM) [COVERING ANY OF ITS INDIVIDUAL ANTI- AND SYN-ISOMERS OR ANY COMBINATION THEREOF]

AS A SUBSTANCE OF VERY HIGH CONCERN BECAUSE OF ITS vPvB[1] PROPERTIES (ARTICLE 57(E))

Adopted on 30 November 2017

[1] vPvB means very persistent and very bioaccumulative

CONTENTS

IDENTIFICATION OF A SUBSTANCE OF VERY HIGH CONCERN ON THE BASIS OF THE CRITERIA SET OUT IN REACH ARTICLE 57 5

JUSTIFICATION 7

IDENTITY OF THE SUBSTANCE AND PHYSICAL AND CHEMICAL PROPERTIES 8

1.1 NAME AND OTHER IDENTIFIERS OF THE SUBSTANCE 8
1.3 IDENTITY AND COMPOSITION OF DEGRADATION PRODUCTS/METABOLITES RELEVANT FOR THE SVHC ASSESSMENT 12
1.4 IDENTITY AND COMPOSITION OF STRUCTURALLY RELATED SUBSTANCES (USED IN A BENCHMARKING OR READ-ACROSS APPROACH) 16
1.5 PHYSICOCHEMICAL PROPERTIES 22

2 HARMONISED CLASSIFICATION AND LABELLING 25

3 ENVIRONMENTAL FATE PROPERTIES 25

3.1 DEGRADATION 25
3.1.1 Abiotic degradation 25
3.1.2 Biodegradation 26
3.1.3 Other data 31
3.1.4 Summary and discussion of degradation 31
3.2 ENVIRONMENTAL DISTRIBUTION 32
3.2.1 Adsorption/desorption 32
3.2.2 Volatilisation 32
3.2.3 Distribution modelling 33
3.3 DATA INDICATING POTENTIAL FOR LONG-RANGE TRANSPORT 33
3.4 BIOACCUMULATION 35
3.4.1 Bioaccumulation in aquatic organisms (pelagic & sediment organisms) 35
3.4.2 Bioaccumulation in terrestrial organisms (soil dwelling organisms, vertebrates) 49
3.4.4 Summary and discussion of bioaccumulation 57

4 HUMAN HEALTH HAZARD ASSESSMENT 59

4.1 TOXICOKINETICS (ABSORPTION, METABOLISM, DISTRIBUTION AND ELIMINATION) 59

5 ENVIRONMENTAL HAZARD ASSESSMENT 61

6 CONCLUSIONS ON THE SVHC PROPERTIES 62

6.1 CMR ASSESSMENT 62
6.2 PBT AND VPVB ASSESSMENT 62
6.2.1 Assessment of PBT/vPvB properties 62
6.2.1.3.1 Fulfilment of the T criterion based on human health classification 67
6.2.1.3.2 Fulfilment of the T criterion based on ecotoxicity data 67
6.2.2 Summary and overall conclusions on the PBT and vPvB properties 69

REFERENCES FOR MAIN REPORT AND APPENDICES 71

APPENDIX 1 DETAILED SUMMARIES OF DATA CITED IN THE MAIN REPORT 86

1 IDENTITY OF THE SUBSTANCE AND PHYSICAL AND CHEMICAL PROPERTIES 88

2 CLASSIFICATION AND LABELLING 88

3 ENVIRONMENTAL FATE PROPERTIES 88

3.1 DEGRADATION 88
3.1.1 Abiotic degradation 88
3.1.2 Biodegradation 90
3.2 ENVIRONMENTAL DISTRIBUTION 96
3.2.1 Adsorption/desorption 96

3.2.2 Volatilisation 97
3.3 Potential for long range transport 97
3.4 Bioaccumulation 103
3.4.1 Bioaccumulation in aquatic organisms (pelagic and sediment organisms) 103
3.4.2 Bioaccumulation in terrestrial organisms (soil-dwelling organisms, vertebrates) 147

4 HUMAN HEALTH HAZARD ASSESSMENT RELEVANT FOR THE PBT ASSESSMENT 163

4.1 Toxicokinetics 163
4.2 Repeated dose toxicity 163
4.2.1 Repeated dose toxicity: oral 163
4.2.2 Repeated dose toxicity: inhalation 164
4.2.3 Repeated dose toxicity: dermal 164
4.2.4 Summary and discussion of repeated dose toxicity 164
4.3 Mutagenicity 165
4.3.1 In vitro data 165
4.3.2 In vivo data 165
4.3.3 Summary and discussion of mutagenicity 165
4.4 Carcinogenicity 166
4.4.1 Summary and discussion of carcinogenicity 166
4.5 Toxicity to reproduction 166
4.5.1 Effects on fertility and development 166
4.5.2 Summary and discussion of toxicity to reproduction 166
4.6 Summary of mammalian toxicity 166

5 ENVIRONMENTAL HAZARD ASSESSMENT 167

5.1 Aquatic compartment (including sediment) 167
5.1.1 Pelagic toxicity 167
5.1.2 Sediment organisms 180
5.2 Terrestrial compartment 181
5.3 Atmospheric compartment 185
5.4 Microbiological activity in sewage treatment systems 185
5.4.1 Toxicity to aquatic micro-organisms 185
5.5 Non compartment specific effects relevant for the food chain (secondary poisoning) 185
5.5.1 Toxicity to birds 185
5.5.2 Toxicity to mammals 188

6 MONITORING DATA 188

ANNEX LITERATURE SEARCH 189

APPENDIX 2 TRANSFORMATION PRODUCTS 196

APPENDIX 3 MEASURED CONCENTRATIONS OF DECHLORANE PLUS IN BIOTA ASSOCIATED WITH THE AQUATIC ENVIRONMENT 205

APPENDIX 4 MEASURED CONCENTRATIONS OF DECHLORANE PLUS IN BIOTA ASSOCIATED WITH THE TERRESTRIAL ENVIRONMENT 233

APPENDIX 5 OTHER EUROPEAN MONITORING STUDIES 246

APPENDIX 6 COMPARISON OF LABORATORY BIOCONCENTRATION DATA BETWEEN SUBSTANCES 250

APPENDIX 7 BENCHMARKING OF BIOACCUMULATION USING POLYCHLOROBIPHENYLS (PCBS) 256

APPENDIX 8 PREDICTED AQUATIC TOXICITY OF SELECTED DECHLORANE PLUS ANALOGUES USING EPISUITE V4.11 258

APPENDIX 9 FURTHER REFERENCES THAT APPEAR RELEVANT BUT ARE NOT INCLUDED IN THE ASSESSMENT 260

TABLES

Table 1: Substance identity of 1,6,7,8,9,14,15,16,17,17,18,18-dodecachloropentacyclo-[12.2.1.1^{6,9}.0^{2,13}.0^{5,10}]octadeca-7,15-diene, Dechlorane Plus [1]....8
Table 2**:** Substance identity of (1S,2S,5S,6S,9R,10R,13R,14R)-1,6,7,8,9,14,15,16,17,17, 18,18-dodecachloropentacyclo[12.2.1.1^{6,9}.0^{2,13}.0^{5,10}]octadeca-7,15-diene, anti- (or exo) Dechlorane Plus [2, see structural formula below] 9
Table 3**:** Substance identity of (1S,2S,5R,6R,9S,10S,13R,14R)-1,6,7,8,9,14,15,16,17,17, 18,18-dodecachloropentacyclo[12.2.1.1^{6,9}.0^{2,13}.0^{5,10}]octadeca-7,15-diene, syn- (or endo) Dechlorane Plus [3, , see structural formula below]....10
Table 4: Constituents other than impurities/additives....11
Table 4: Identity of 1,3-DPMA 13
Table 4: Identity of 1,5-DPMA....14
Table 4: Identity of DP-1Cl....15
Table 4: Identity of DP-2Cl 15
Table 5: Identity of selected analogues 18
Table 6: Overview of physicochemical properties 22
Table 7: Summary of predicted and measured degradation data for Dechlorane Plus analogues 29
Table 8: Input properties used in assessing long range transport potential....34
Table 9: Depuration half-lives and BCF values for chemicals meeting the vB criteria 44

FIGURES

Figure 1: Geometric isomers of Dechlorane Plus 11
Figure 2: Potential dechlorinated transformation products of Dechlorane Plus 12
Figure 3: Potential mono-adduct transformation products of Dechlorane Plus 13
Figure 4: Dechlorane Plus levels in chicken tissues 53

IDENTIFICATION OF A SUBSTANCE OF VERY HIGH CONCERN ON THE BASIS OF THE CRITERIA SET OUT IN REACH ARTICLE 57

Substance Names: 1,6,7,8,9,14,15,16,17,17,18,18-Dodecachloropentacyclo-[12.2.1.1^{6,9}.0^{2,13}.0^{5,10}]octadeca-7,15-diene ("Dechlorane Plus"^{TM})

[covering any of its individual anti- and syn-isomers or any combination thereof]

EC Numbers: 236-948-9; -; -

CAS numbers: 13560-89-9; 135821-74-8; 135821-03-3

- The substance is identified as very persistent and very bioaccumulative (vPvB) according to Article 57(e) of Regulation (EC) No 1907/2006 (REACH).

Summary of how the substance meets the criteria set out in Article 57 of the REACH Regulation

Persistence

Based on the weight of evidence of the data available, it is concluded that Dechlorane Plus meets the criteria for vP in Annex XIII of REACH. This is based on:

- modelling of degradation potential and microbial metabolic pathways which suggests that biodegradation is likely to be very slow; and

- a low probability that it will degrade any faster than structural analogues that are considered to be very persistent under the Stockholm Convention.

This conclusion is also supported by the very low water solubility (suggesting limited bioavailability to micro-organisms once bound to solid matrices), monitoring data indicating that Declorine Plus can persist in sediments (a major sink) for many years, lack of evidence of biotransformation in fish (supporting the premise that the molecule is metabolically recalcitrant) and widespread occurrence in remote regions.

Bioaccumulation

Using a weight of evidence assessment of the data available, Dechlorane Plus meets the vB criteria in Annex XIII of REACH. This is based on:

- the long-depuration half-life determined in fish feeding studies which is indicative of a BCF above 5 000 L/kg, by comparison with other substances (supported by a long depuration half-life in mammalian liver);

- numerous studies that show that the substance is widely dispersed in freshwater, marine and terrestrial food chains, including top predators; and

- evidence that the substance can exceed levels in biota that are of concern based on critical body burden considerations related to baseline narcosis.

This conclusion is supported by the detection of Declorine Plus in human blood,

placenta and breast milk.

Toxicity

Based on the available ecotoxicity and mammalian data, Dechlorane Plus does not currently meet the T criterion. Long-term toxicity studies using relevant life stages of fish (via diet), sediment or soil organisms, and/or birds could be performed to clarify whether adverse effects can occur via these exposure routes. However, as Declorine Plus meets both the vP and vB criteria, these are not scientifically necessary for environmental risk management purposes.

Other concerns

The substances 1,3- and 1,5-Dechlorane Plus monoadduct (DPMA) have been detected in the environment, sometimes at higher concentrations than Dechlorane Plus in the same samples. DPMA might be under-reported because destructive sample preparation methods may degrade it. Dechlorane Plus is the only likely source of these two substances, although there is no information on reaction rates or amounts that can be formed under relevant environmental conditions. Based on predictive models, DPMA screens as being potentially PBT and vPvB on the basis of QSAR (although some of the predictions are uncertain). No information is available on its mammalian toxicity, but due to structural similarities to aldrin or heptachlor it might be epoxidised in the environment to form a substance that could be neurotoxic and/or cause hepatotoxicity. Experimental data would be needed to confirm these properties. However, as a degradation product of Dechlorane Plus, any concerns about DPMA would be alleviated by the identification of Dechlorane Plus as a substance of very high concern.

In conclusion, despite the lack of definitive data, Dechlorane Plus is identified as a vPvB substance according to Art. 57(e) of REACH by comparing all relevant and available information listed in Annex XIII of REACH with the criteria set out in the same Annex, in a weight-of-evidence determination.

Registration dossiers submitted for the substance: Yes

Justification

The data described in this dossier were taken from public sources unless stated otherwise, including robust study summaries from the REACH registration dossier available on the ECHA dissemination website on 24th August 2017 (http://www.echa.europa.eu/) and submitted to the U.S. EPA by the Occidental Chemical Company (2003). Studies that are not formal regulatory test reports or published in peer reviewed journals (e.g. conference posters) are indicated as abstracts only [ABST]. Original test reports have not been reviewed (unless stated), although the information in the robust study summaries is considered sufficient. Published literature articles have been reviewed in detail.

The number of published papers is growing at a fast rate and it is not considered proportionate to attempt to summarise all available studies that refer to this substance. Detailed study descriptions for the major end points relevant to this dossier are provided in Appendix 1, which also includes a list of references that are not considered relevant, and another list of references that might be relevant but are likely to be of relatively low significance.

Identity of the substance and physical and chemical properties

1.1 Name and other identifiers of the substance

The substance 1,6,7,8,9,14,15,16,17,17,18,18-dodecachloropentacyclo-[12.2.1.1^{6,9}.0^{2,13}.0^{5,10}]octadeca-7,15-diene has two isomers, named anti- [2] and syn- [3]. This dossier covers the individual anti- and syn- isomers (mono-constituent substances) and all possible combinations of the syn- and anti- isomers [1] (see structural formula below)**.**

Table 1: Substance identity of 1,6,7,8,9,14,15,16,17,17,18,18-dodecachloropentacyclo-[12.2.1.1^{6,9}.0^{2,13}.0^{5,10}]octadeca-7,15-diene, Dechlorane Plus [1].

EC number:	236-948-9
EC name:	1,6,7,8,9,14,15,16,17,17,18,18-Dodecachloro-pentacyclo[12.2.1.1^{6,9}.0^{2,13}.0^{5,10}]octadeca-7,15-diene
CAS number (in the EC inventory):	13560-89-9
CAS number: **Deleted CAS numbers:**	13560-89-9 -
CAS name:	1,4:7,10-Dimethanodibenzo[*a*,*e*]cyclooctene, 1,2,3,4,7,8,9,10,13,13,14,14-dodecachloro-1,4,4a,5,6,6a,7,10,10a,11,12,12a-dodecahydro-
IUPAC name:	1,6,7,8,9,14,15,16,17,17,18,18-Dodecachloropentacyclo[12.2.1.1^{6,9}.0^{2,13}.0^{5,10}]octadeca-7,15-diene
Index number in Annex VI of the CLP Regulation	Not applicable
Molecular formula:	$C_{18}H_{12}Cl_{12}$
Molecular weight range:	653.73 g/mole
Synonyms:	Bis(hexachlorocyclopentadieno)cyclooctane; 1,2,3,4,7,8,9,10,13,13,14,14-Dodecachloro-1,4,4a,5,6,6a,7,10,10a,11,12,12a-dodechydro-1,4:7,10-dimethanodibenzo[a,e]cyclooctene; Dodecachlorododecahydrodimethanodibenzocyclooctene; Dechlorane Plus 25 (Dech Plus); Dechlorane Plus 35 (Dech Plus-2); DP-515; Dechlorane 605; DP; DDC-CO

Note: The academic literature usually refers to this substance by a registered trade name "Dechlorane Plus" (often abbreviated as DP, but sometimes DDC-CO), and this is the name used throughout this report for convenience.

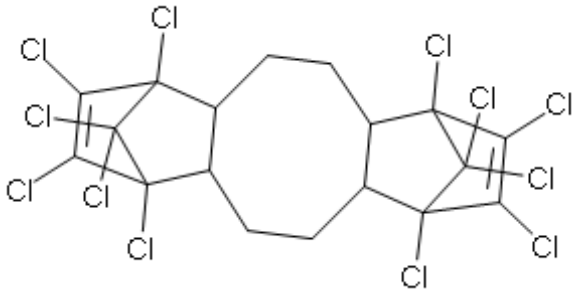
Table 2**:** Substance identity of (1S,2S,5S,6S,9R,10R,13R,14R)-1,6,7,8,9,14,15,16,17,17,18,18-dodecachloropentacyclo[12.2.1.$1^{6,9}$.$0^{2,13}$.$0^{5,10}$]octadeca-7,15-diene, anti- (or exo) Dechlorane Plus [2, see structural formula below]

EC number:	-
EC name:	-
CAS number: **Deleted CAS numbers:**	135821-74-8 -
CAS name:	1,4:7,10-Dimethanodibenzo[*a*,*e*]cyclooctene, 1,2,3,4,7,8,9,10,13,13,14,14-dodecachloro-1,4,4a,5,6, 6a,7,10,10a,11,12,12a-dodecahydro-, (1*R*,4*S*,4a*S*,6a*S*,7*S*,10*R*,10a*R*,12a*R*)-*rel*-
IUPAC name:	(1S,2S,5S,6S,9R,10R,13R,14R)-1,6,7,8,9,14,15,16,17,17,18,18-Dodecachloropentacyclo[12.2.1.$1^{6,9}$.$0^{2,13}$.$0^{5,10}$]octadeca-7,15-diene
Index number in Annex VI of the CLP Regulation	Not applicable
Molecular formula:	$C_{18}H_{12}Cl_{12}$
Molecular weight range:	653.73 g/mole
Synonyms:	anti-DP, anti-Dechlorane plus, anti-Dodecachloropentacyclooctadecadiene

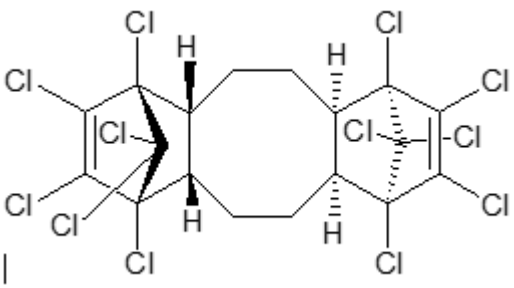
Table 3**:** Substance identity of (1S,2S,5R,6R,9S,10S,13R,14R)-1,6,7,8,9,14,15,16,17,17,18,18-dodecachloropentacyclo[12.2.1.$1^{6,9}$.$0^{2,13}$.$0^{5,10}$]octadeca-7,15-diene, syn- (or endo) Dechlorane Plus [3, , see structural formula below]

EC number:	-
EC name:	-
CAS number: **Deleted CAS numbers:**	135821-03-3 -
CAS name:	1,4:7,10-Dimethanodibenzo[a,e]cyclooctene, 1,2,3,4,7,8,9,10,13,13,14,14-dodecachloro-1,4,4a,5,6,6a,7,10,10a,11,12,12a-dodecahydro-, (1*R*,4*S*,4a*S*,6a*R*,7*R*,10*S*,10a*S*,12a*R*)-*rel*-
IUPAC name:	(1S,2S,5R,6R,9S,10S,13R,14R)-1,6,7,8,9,14,15,16,17,17,18,18-Dodecachloropentacyclo[12.2.1.$1^{6,9}$.$0^{2,13}$.$0^{5,10}$]octadeca-7,15-diene
Index number in Annex VI of the CLP Regulation	Not applicable
Molecular formula:	$C_{18}H_{12}Cl_{12}$
Molecular weight range:	653.73 g/mole
Synonyms:	syn-DP, syn-Dechlorane plus, syn-Dodecachloropentacyclooctadecadiene

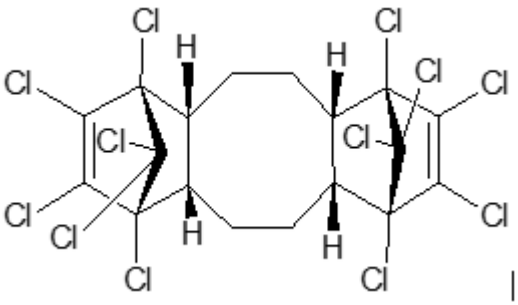
Structural formula:



anti- (or exo) Dechlorane Plus [2]



syn- (or endo) Dechlorane Plus [3]



1.2 Composition of the substance

Name: Dechlorane Plus™

Substance type: not applicable (group entry)

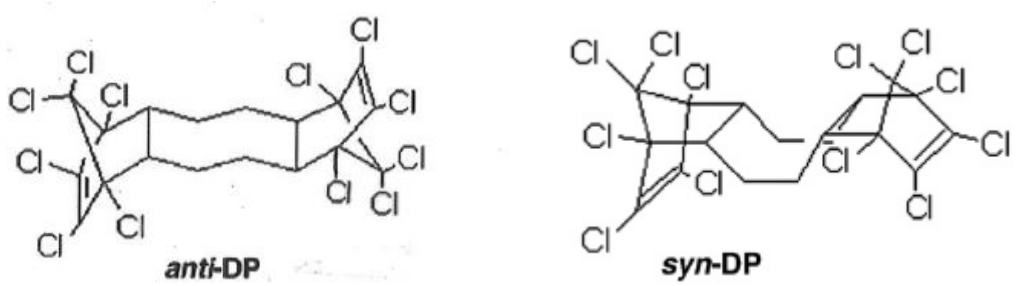
The information in this section is for the substance containing both the anti- and the syn- isomers as main constituents.

Table 4: Constituents other than impurities/additives

Constituents	Typical concentration	Concentration range (w/w)	Reference
anti- (or exo-)Dechlorane Plus (CAS no. 135821-74-8)	-	60-80 %	Ben *et al*. (2013)
syn- (or endo-)Dechlorane Plus (CAS no. 135821-03-3)	-	20-40 %	Ben *et al*. (2013)

The substance is described as mono-constituent by the lead Registrant. However, two geometric isomers are present in the commercial substance (e.g. Chou *et al*., 1979; Occidental, 2013). This means that it is multi-constituent. The structures of the two isomers are provided in Figure 1.

Figure 1: Geometric isomers of Dechlorane Plus



(reprinted from Muñoz-Arnanz *et al*. (2010). Copyright 2010: International Symposium on Halogenated Persistent Organic Pollutants)

Ben *et al.* (2013) reported that the anti- isomer fractional abundance (f_{anti}) value (defined as [anti- isomer]/([anti- isomer] + [syn- isomer])) is not constant in Chinese commercial products, and varies from 0.60 to 0.80. The f_{anti} value of OxyChem commercial products has also been reported by several authors to be in the range 0.64 to 0.80 (e.g. see references in Wang *et al*., 2010).

The substance is made by a Diels-Alder reaction between 1,5-cyclooctadiene and hexachlorocyclopentadiene in a molar ratio of 2:1. Cyclooctadiene can also exist as 1,4- and 1,3- isomers, and both these, 4-vinylcyclohexene and 1,2-divinylcyclobutane might be present as impurities in, or formed via thermal rearrangement of, the starting materials (Sverko *et al*., 2010b). Consequently, they can produce Diels-Alder reaction products with the same molecular weight as Dechlorane Plus. Sverko *et al*. (2010b) analysed a technical Dechlorane Plus product and detected four minor chromatographic peaks that are potentially related to these other substances.

Compounds with a smaller number of chlorine atoms may also be impurities in the commercial substance. For example, Li *et al*. (2013b) found a mono-dechlorinated substance (DP-1Cl; see Section 1.3) in the commercial substance produced by Jiangsu Anpon Co. Ltd., China; in contrast, Peng *et al*. (2014) could not detect

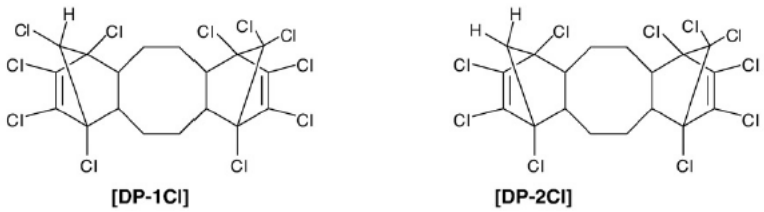
DP-1Cl in samples from the same source (although this might reflect differences in detection limits).

Based on the variation of isomer-specific properties and the available test results, the study outcomes reported in Sections 3 can be considered to reflect with sufficient certainty the P and B properties of this group entry. The Annex XV entry covers the identity of the assessed substance to the level possible.

1.3 Identity and composition of degradation products/metabolites relevant for the SVHC assessment

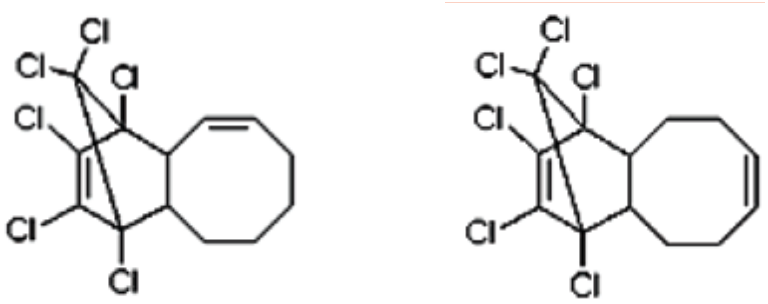
Three substances are relevant to this evaluation because they have been detected in various monitoring studies (e.g. Sverko *et al*., 2008 & 2010b; Muñoz-Arnanz *et al*., 2010; Möller *et al*., 2010; Guerra *et al*., 2011; Sun *et al*., 2012; Chen *et al*., 2013b; Yu *et al*., 2013; Ben *et al*., 2013 & 2014; Zheng *et al*., 2014a; Wang *et al*., 2015). These are 1,3-Dechlorane Plus monoadduct (1,3-DPMA), 1,5-Dechlorane Plus monoadduct (1,5-DPMA) and two substances formed through dehalogenation: one that has a single chlorine atom replaced by hydrogen (DP-1Cl or Cl_{11}-DP) and another in which two adjacent chlorine atoms are replaced by hydrogen (DP-2Cl or Cl_{10}-DP). The structures of these three substances are provided in Figure 2. Other substances could include hydroxyl or other substituents in place of the hydrogen atoms.

Figure 2: Potential dechlorinated transformation products of Dechlorane Plus



(reprinted from Muñoz-Arnanz *et al*. (2010). Copyright 2010: International Symposium on Halogenated Persistent Organic Pollutants)

Figure 3: Potential mono-adduct transformation products of Dechlorane Plus



1,3-DPMA **1,5-DPMA**

(adapted with permission from Sverko *et al*. (2010). Copyright 2010: American Chemical Society)

There is no information about their rates of formation or the conditions under which they are formed. They might also be impurities in the commercial substance (see Section 1.2). Their properties have been estimated for the purposes of this document (see Appendix 2).

Table 4: Identity of 1,3-DPMA

EC number:	Not available
EC name:	Not available
SMILES:	C32(Cl)C(Cl)(Cl)C(Cl)(C(Cl)=C3Cl)C1C2CCCCC=C1
CAS number (in the EC inventory):	Not available
CAS number:	Not available
CAS name:	Not available
IUPAC name:	1,10,11,12,13,-13-Hexachlorotricyclo[8.21.$0^{2,9}$]-trideca-3,11-diene
Index number in Annex VI of the CLP Regulation	Not applicable
Molecular formula:	$C_{13}H_{12}Cl_6$
Molecular weight range:	380.96 g/mole
Synonyms:	1,3-DPMA, 1,3-Dechlorane Plus mono-adduct

Table 4: Identity of 1,5-DPMA

EC number:	Not available
EC name:	Not available
SMILES:	C32(Cl)C(Cl)(Cl)C(Cl)(C(Cl)=C3Cl)C1C2CCC=CCC1
CAS number (in the EC inventory):	Not available
CAS number:	Not available
CAS name:	Not available
IUPAC name:	1,10,11,12,13,-13-Hexachlorotricyclo[8.21.$0^{2,9}$]-trideca-5,11-diene
Index number in Annex VI of the CLP Regulation	Not applicable
Molecular formula:	$C_{13}H_{12}Cl_6$
Molecular weight range:	380.96 g/mole
Synonyms:	1,5-DPMA, 1,5-Dechlorane Plus mono-adduct

Table 4: Identity of DP-1Cl

EC number:	Not available
EC name:	Not available
SMILES:	C(=C(C(C1(CL)CL)(C(C2CCC(C(C(=C(C34CL)CL)CL)(C3(H)CL)CL)C4C5)C5)CL)CL)(C12CL)CL
CAS number (in the EC inventory):	Not available
CAS number:	Not available
CAS name:	Not available
IUPAC name:	1,6,7,8,9,14,15,16,17,17,18-Undecachloropenta-cyclo[12.2.1.1^{6,9}.0^{2,13}.0^{5,10}]octadeca-7,15-diene
Index number in Annex VI of the CLP Regulation	Not applicable
Molecular formula:	$C_{18}H_{13}Cl_{11}$
Molecular weight range:	619.29 g/mole
Synonyms:	DP-1Cl or Cl_{11}-DP

Table 4: Identity of DP-2Cl

EC number:	Not available
EC name:	Not available
SMILES:	C(=C(C(C1(CL)CL)(C(C2CCC(C(C(=C(C34CL)CL)CL)(C3(H)H)CL)C4C5)C5)CL)CL)(C12CL)CL
CAS number (in the EC inventory):	Not available
CAS number:	Not available
CAS name:	Not available
IUPAC name:	1,6,7,8,9,14,15,16,17,17-Decachloropenta-cyclo[12.2.1.1^{6,9}.0^{2,13}.0^{5,10}]octadeca-7,15-diene
Index number in Annex VI of the CLP Regulation	Not applicable
Molecular formula:	$C_{18}H_{14}Cl_{10}$
Molecular weight range:	584.84 g/mole
Synonyms:	DP-2Cl or Cl_{10}-DP

1.4 Identity and composition of structurally related substances (used in a benchmarking or read-across approach)

Ten substances sharing the hexachlorinated norbornene moiety are listed in Table 5 along with data for key physico-chemical end points.[2] The measured data are taken from the 13th (Budavari, 2001) and 15th (O'Neil, 2013) editions of *The Merck Index* (and subsequently https://www.rsc.org/Merck-Index/) and the experimental database within *EPI Suite v4.11* (U.S. EPA, 2012). Data were estimated using *EPI Suite v4.11* for consistency reasons.

Dechlorane Plus contains two chlorinated norbornene groups, whereas seven of the potential analogues only contain one such group. Whilst they tend to share limited water solubility, high n-octanol-water partition coefficients (log K_{OW} values) and very low vapour pressures, they are not a homogeneous group:

- Compared to Dechlorane Plus, six of the substances (dieldrin/endrin, aldrin, endosulfan, chlorendic acid[3], Dechlorane 602 and Dechlorane 604) contain additional functional groups (i.e. epoxide, alkene, sulfite, carboxylic acid, furan or brominated benzene) that affect polarity and reactivity, and are likely to influence their (eco)toxicological and environmental fate properties. With the exception of Dechlorane 602 and Dechlorane 604, they also have a lower molecular weight and higher water solubility than Dechlorane Plus. Therefore, these substances are not suitable analogues for Dechlorane Plus in terms of bioaccumulation or (eco)toxicity assessment, but they may offer a 'best case' comparison in terms of persistence.

- Chlordane and heptachlor do not contain reactive functional groups but do have additional chlorine atoms, so are closer structural analogues to Dechlorane Plus. However, they have much lower molecular weights (410 and 370 g/mole, respectively, compared to 650 g/mole for Dechlorane Plus), significantly higher water solubility (0.06 and 0.2 mg/L, respectively, compared to <0.00000167 mg/L for Dechlorane Plus), and lower log K_{OW} values (6.3 and 5.9, respectively, compared to ≥9 for Dechlorane Plus). Their bioavailability and toxicokinetic profile are likely to be quite different to Dechlorane Plus and so direct read-across of bioaccumulation and (eco)toxicity end points is not appropriate. Read-across for persistence is likely to be more reliable than for the previous six substances.

- Dechlorane 603 and Chlordene Plus have two chlorinated norbornene groups like Dechlorane Plus and no additional reactive functional groups. They have lower conformational flexibility, which could affect reactivity. They have similar molecular weights to Dechlorane Plus (611 – 638 g/mole), and very similar predicted water solubility and log K_{OW} values. They are therefore its closest structural analogues. They appear to have

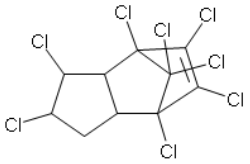
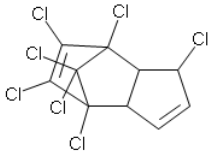
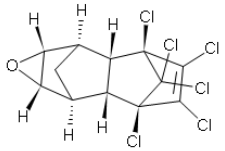
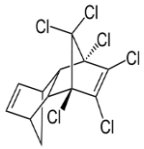
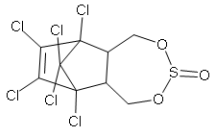
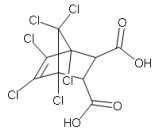
[2] Mirex (EC no. 219-196-6, CAS no. 2385-85-5) [also known as Dechlorane and perchloropentacyclodecane] is made using similar starting materials, but has a much more compact carbon skeleton and is fully chlorinated (molecular formula: $C_{10}Cl12$; SMILES: C12(C3(C4(C5(C3(C(C1(C5(C2(C4(Cl)Cl)Cl)Cl)Cl)(Cl)Cl)Cl)Cl)Cl)Cl)Cl). It is therefore not a suitable analogue for the purposes of this assessment. Further information can be found in WHO (1984b & 1995).

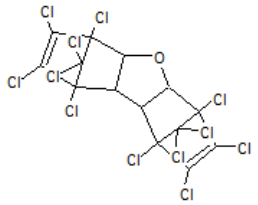
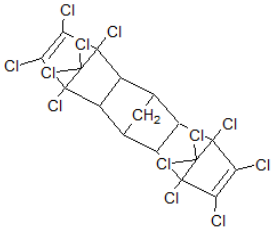
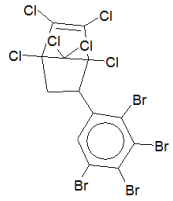
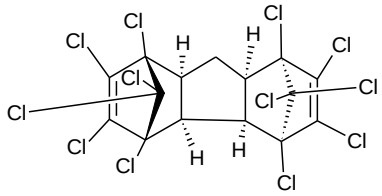
[3] Chlorendic anhydride (EC no. 204-077-3, CAS no. 115-27-5) is another analogue but as it rapidly forms chlorendic acid in contact with water it is not directly relevant for this analysis. The acid was considered as part of an EU Substance Evaluation of chlorendic anhydride. A decision requesting further information was sent to the Registrant(s) and the data submission deadline was 26 September 2016. The follow-up evaluation is not yet available.

been impurities in some pesticide active substances rather than commercial products as such, and no experimental data seem to be available.

Table 5: Identity of selected analogues

Substance name	Chlordane[a]	Heptachlor[b]	Dieldrin[c]	Aldrin[d]	Endosulfan	Chlorendic acid
EC number:	200-349-0	200-962-3	200-484-5	206-215-8	204-079-4	
EC name:	Chlordane , pur	Heptachlor	Dieldrin	Aldrin	Endosulfan	1,4,5,6,7,7-hexachloro-8,9,10-trinorborn-5-ene-2,3-dicarboxylic acid
Canonical SMILES	ClC1CC2C(C1Cl)C1(C(C2(Cl)C(=C1Cl)Cl)(Cl)Cl)Cl	ClC1C=CC2C1C1(Cl)C(Cl)=C(Cl)C2(Cl)C1(Cl)Cl	ClC4=C(Cl)[C@]5(Cl)[C@H]1[C@H]([C@H]3C[C@@H]1[C@@H]2O[C@@H]23)[C@@]4(Cl)C5(Cl)Cl ClC4=C(Cl)[C@@]5(Cl)[C@@H]1[C@@H]([C@@H]3C[C@H]1[C@H]2O[C@H]23)[C@]4(Cl)C5(Cl)Cl	ClC3=C(Cl)[C@@]4(Cl)[C@@H]2[C@@H]([C@@H]1C[C@H]2C=C1)[C@]3(Cl)C4(Cl)Cl ClC3=C(Cl)[C@]4(Cl)[C@H]2[C@H]([C@H]1C[C@@H]2C=C1)[C@@]3(Cl)C4(Cl)Cl	O=S1OCC2C(CO1)C1(C(C2(Cl)C(=C1Cl)Cl)(Cl)Cl)Cl	OC(=O)C1C(C(=O)O)C2(C(C1(Cl)C(=C2Cl)Cl)(Cl)Cl)Cl
CAS number (in the EC inventory):	57-74-9	76-44-8	60-57-1	309-00-2	115-29-7	115-28-6
CAS no.	57-74-9	76-44-8	60-57-1	309-00-2	115-29-7	115-28-6
CAS name:	4,7-Methano-1H-indene, 1,2,4,5,6,7,8,8-octachloro-2,3,3ª,4,7,7ª-hexahydro-	4,7-Methano-1H-indene, 1,4,5,6,7,8,8-heptachloro-3ª,4,7,7ª-tetrahydro-	2,7:3,6-Dimethanonaphth[2,3-b]oxirene, 3,4,5,6,9,9-hexachloro-1ª,2,2ª,3,6,6ª,7,7ª-octahydro-, (1ªR,2R,2ªS,3S,6R,6ªR,7S,7ªS)-rel-	1,4:5,8-Dimethanonaphthalene, 1,2,3,4,10,10-hexachloro-1,4,4ª,5,8,8ª-hexahydro-, (1R,4S,4ªS,5S,8R,8ªR)-rel-	6,9-Methano-2,4,3-benzodioxathiepin, 6,7,8,9,10,10-hexachloro-1,5,5ª,6,9,9ª-hexahydro-, 3-oxide	Bicyclo[2.2.1]hept-5-ene-2,3-dicarboxylic acid, 1,4,5,6,7,7-hexachloro-
IUPAC name:	1,2,4,5,6,7,8,8-Octachloro-3ª,4,7,7ª-tetrahydro-4,7-methanoindane	1,4,5,6,7,8,8-Heptachloro-3ª,4,7,7a-tetrahydro-4,7-methano-1*H*-indene	rel-(1S,2R,3R,6S,7S,8R,9R,11S)-3,4,5,6,13,13-hexachloro-10-oxapentacyclo[6.3.1.1³,⁶.0²,⁷.0⁹,¹¹]tridec-4-ene	rel-(1R,2R,3R,6S,7S,8S)-1,8,9,10,11,11-hexachlorotetracyclo[6.2.1.1^{3,6}.0^{2,7}]dodeca-4,9-diene	1,2,3,4,7,7-hexachloro-8,9,10-trinorborn-2-en-5,6-ylenedimethylene sulfite|1,4,5,6,7,7-hexachloro-8,9,10-trinorborn-5-en-2,3-ylenedimethylene sulfite	1,4,5,6,7,7-hexachloro-5-norbornene-2,3-dicarboxylic acid
Index no. in CLP Annex VI	602-047-00-8	602-046-00-2	602-049-00-9	602-048-00-3	602-052-00-5	-
Molecular formula:	$C_{10}H_6Cl_8$	$C_{10}H_5Cl_7$	$C_{12}H_8Cl_6O$	$C_{12}H_8Cl_6$	$C_9H_6Cl_6O_3S$	$C_9H_4Cl_6O_4$

Structural formula						
Molecular weight, g/mole	409.78	373.32	380.91	364.92	406.93	388.85
Melting point (experimental)	106 °C	95.5 °C	226-230 °C	104 °C, 240°C	106 °C	209 °C
Boiling point (experimental)	175 °C at 2 mm Hg	310 °C	-	-	-	-
Vapour pressure	0.0013 Pa at 25 °C (experimental) 0.003 Pa at 25 °C (estimated)	0.053 Pa at 25 °C (experimental) 0.03 Pa at 25 °C (estimated)	4.0E-04 Pa at 20 °C (experimental) 3.7 E-04 Pa at 25 °C (estimated)	0.016 Pa at 25 °C (experimental) 2.5 E-04 Pa at 25 °C (estimated)	8.0E-05 Pa at 25 °C (experimental) 1.7E-04 Pa at 25 °C (estimated)	1.9E-06 Pa at 25 °C (estimated)
Water solubility	0.056 mg/L at 25 °C (experimental) 0.013 mg/L at 25 °C (estimated)	0.18 mg/L at 25 °C (experimental) 0.095 mg/L at 25 °C (estimated)	0.195 - 0.25 mg/L at 25 °C (experimental) 0.15 mg/L at 25 °C (estimated)	0.017 mg/L at 25 °C (experimental) 0.002 - 0.014 mg/L at 25 °C (estimated)	0.325 mg/L at 22 °C (experimental) 1.5 mg/L at 25 °C (estimated)	3,500 mg/L at 25 °C (experimental) 18 mg/L at 25 °C (estimated)
log K_{ow}	6.1-6.2 (experimental) 6.3 (estimated)	5.5-6.1 (experimental) 5.9 (estimated)	5.2-5.4 (experimental) 5.4 (estimated)	6.5 (experimental) 6.8 (estimated)	3.8 (experimental) 2.25 (estimated)	3.1 (estimated)

Substance name	Dechlorane 602	Dechlorane 603	Dechlorane 604	Chlordene Plus
EC number:	250-472-9	-	252-097-6	-
EC name:	1,2,3,4,6,7,8,9,10,10,11,11-Dodecachloro-1,4,4a,5a,6,9,9a,9b-octahydro-1,4:6,9-dimethanodibenzofuran		1,2,3,4,7,7-Hexachloro-5-(tetrabromophenyl)bicyclo[2.2.1]hept-2-ene	
Canonical SMILES	C12C3C(C4(C(=C(C3(C4(Cl)Cl)Cl)Cl)Cl)Cl)OC1C5(C(=C(C2(C5(Cl)Cl)Cl)Cl)Cl)Cl	C62(Cl)C(Cl)(Cl)C(Cl)(C(Cl)=C6Cl)C1C3C4C5(Cl)C(Cl)(Cl)C(Cl)(C(Cl)=C5Cl)C4C(C21)C3	C1C(C2(C(=C(C1(C2(Cl)Cl)Cl)Cl)Cl)Cl)C3=CC(=C(C(=C3Br)Br)Br)Br	ClC4=C(Cl)[C@@]5(Cl)[C@@H]1[C@@H](C[C@H]2[C@@H]1[C@]3(Cl)C(Cl)=C(Cl)[C@@]2(Cl)C3(Cl)Cl)[C@]4(Cl)C5(Cl)Cl
CAS number (in the EC inventory):	31107-44-5	13560-92-4	34571-16-9	13560-91-3
CAS no.	31107-44-5	13560-92-4	34571-16-9	13560-91-3
CAS name:				
IUPAC name:	1,4,5,6,7,11,12,13,14,14,15,15-dodecachloro-9-oxapentacyclo[9.2.1.1⁴,⁷.0²,¹⁰.0³,⁸]pentadeca-5,12-diene	1,2,3,4,5,6,7,8,12,12,13,13-dodecachloro-1,4,4a,5,8,8a,9,9a,10,10a-decahydro-1,4:5,8:9,10-trimethanoanthracene	1,2,3,4,7,7-hexachloro-5-(2,3,4,5-tetrabromophenyl)bicyclo[2.2.1]hept-2-ene	(1*R*,4*S*,4a*R*,4b*S*,5*S*,8*R*,8a*S*,9a*R*)-1,2,3,4,5,6,7,8,10,10,11,11-dodecachloro-4,4a,4b,5,8,8a,9,9a-octahydro-1H-1,4:5,8-dimethanofluorene
Index number in CLP Annex VI	-	-	-	-
Molecular formula:	$C_{14}H_4Cl_{12}O$	$C_{17}H_8Cl_{12}$	$C_{13}H_4Br_4Cl_6$	$C_{15}H_6Cl_{12}$
Structural formula				
Molecular weight, g/mole	613.62	637.69	692.49	611.61
Melting point	190 °C (estimated)	198 °C (estimated)	203 °C (estimated)	189 °C (estimated)
Boiling point	-	-	-	-
Vapour pressure	1.1E-06 Pa at 25 °C (estimated)	3.2E-07 Pa at 25 °C (estimated)	1.7E-07 Pa at 25 °C (estimated)	1.3E-06 Pa at 25 °C (estimated)
Water solubility	≤1.7E-05 mg/L at 25 °C (estimated)	≤6.4E-07 mg/L at 25 °C (estimated)	≤4.7E-06 mg/L at 25 °C (estimated)	6E-07 mg/L at 25 °C (estimated)
log K_{ow}	8.0 (estimated)	11.2 (estimated)	10.6 (estimated)	9.8 (estimated)

Note: a – Chlordane consists of more than 140 compounds, of which trans-chlordane, cis-chlordane and trans-nonachlor are present in the highest amounts (Dearth & Hites, 1991; Liu et al., 2009).

b – Technical heptachlor usually contains about 72 % (±)-heptachlor and 28 % related compounds including about 18 % trans-chlordane (EFSA, 2007).

c – A stereoisomer of dieldrin called endrin (CAS no. 72-20-8) was also made commercially. It has effectively the same physico-chemical properties as dieldrin.

d – A stereoisomer of aldrin called isodrin (CAS no. 465-73-6) was also made commercially. It has effectively the same physico-chemical properties as aldrin.

1.5 Physicochemical properties

Unless otherwise stated, the data are taken from the REACH registration on the ECHA public dissemination website on 24 August 2017. There is no information available for the individual syn- and anti- isomers. Therefore it is not possible to conclude whether there are physicochemical differences between these or not.

Table 65: Overview of physicochemical properties

Property	Value [Unit]	Reference/source of information/remarks
Physical state at 20°C and 101.3 kPa	The substance is a free flowing solid	
Melting/freezing point	Decomposition from 340 – 382 °C (no melting observed)	
Boiling point	*Data waived on the basis of a melting point > 300 °C*	
Vapour pressure	*Data waived on the basis of a melting point > 300 °C*	A vapour pressure of approximately 9.4E-08 Pa at 25 °C is predicted using MPBPVP v1.43 (U.S. EPA, 2012, modified Grain method, recommended for solids). This is highly uncertain (approximately ±1 log unit) as it is close to the lower limit of the range of the model, where there is some scatter in the training set. However, the molecular weight of the substance is within the range of the model's training set. Also, structural analogues are part of the MPBPVP training and test sets. A measured vapour pressure of approximately 0.008 hPa (0.8 Pa) at 200 °C was reported by Occidental Chemical Company (2003). An extrapolated vapour pressure of 4.6E-04 Pa at 25 °C can be estimated from this result using EUSES v2.1.2, and this is preferred for assessment purposes. There is some uncertainty due to the extrapolation from very high temperature, and the unknown reliability of the underlying result. The substance has a very low vapour pressure at environmentally relevant temperatures.
Surface tension	*Data waived on the basis of low water solubility (<1 mg/L).*	
Dissociation constant	*Data waived on the basis of low solubility in water.*	The substance does not contain any acidic or basic functional groups.

Property	Value [Unit]	Reference/source of information/remarks
Water solubility	< 1.67 ng/L at 20 °C (below the limit of quantitation)	Reliability 1: OECD Test Guideline 105 (column elution method) and GLP). Dechlorane Plus (>99 % purity) was coated onto the column using dichloromethane. HPLC grade reagent water was pumped through the column at two different flow rates, and analysed using gas chromatography with micro electron capture detection (GC-ECD). There is some uncertainty in the precise value for water solubility. However, all available measurements and predictions[4] are in agreement that the substance is very poorly water soluble.
Partition coefficient n-octanol/water (log value)	*Waived by Registrant due to low water solubility.*	Chou *et al*. (1979) reported a log K_{OW} of 9.3 (also reported by the U.S. EPA, 2012). This is a calculated value; its validity has not been assessed. A log K_{OW} of 11.3 is predicted using KOWWIN (U.S. EPA, 2012). This result was also reported in the U.S. EPA (2011) review. The predicted result is considered to be within the validity range of the model because the molecular weight of the substance is within the range for this parameter for both the training and test sets. The number of aliphatic chlorines exceeds the maximum occurrences of this fragment in a single compound in the training set (8 in Dechlorane Plus, maximum 6 in the training set). The value is above the log K_{OW} values used in the training and tests sets and above the normal experimental range, but is indicative of the expected lipophilic character of the substance. It would be unusual to expect to quantify values above approximately 9 experimentally. The log of the ratio of n-octanol and water solubilities is >8.4, using a solubility of < 2 ng/L at 20 °C for water and 470 mg/L at 25 °C for n-octanol (see below). Additional estimation methods give similar values. For example, the ACD/Percepta platform gives the following results: LogP Classic: 9.51±0.67; LogP GALAS: 9.16 (Reliability: Borderline; RI = 0.41. Chlordene and different chlordane isomers are in the training set). Whilst there is clearly uncertainty in the value of log K_{OW}, the value is assumed to be ≥9.

[4] Chou *et al*. (1979) reported mean water solubilities of 207 and 572 ng/L for the two isomers at 22±2.5°C using radiolabelled substance in equilibration with water by slow stirring for six weeks. This is considered unreliable by the Registrant. No reason is provided, but the report concluded that samples in the solubility experiment may have contained particulates, and so estimated a solubility of 44.1±2 ng/L at 22 °C (total for both isomers).

Water solubilities estimated based on a log K_{OW} range of 7 to 9 using WSKOWWIN v.1.42 (U.S. EPA, 2012) are 7.5E-05 – 1.5E-06 mg/L [75 – 1.5 ng/L]. The substance is outside the estimation domain of the model because both molecular weight and log K_{OW} are outside the ranges of these parameters in the training and test sets for the method. A water solubility of 6.5E-07 mg/L [0.65 ng/L] can be estimated using the WaterNT v1.01 fragment method (U.S. EPA, 2012), which does not use log K_{OW} as an input. The molecular weight is outside the range of this parameter in the training set, but not the test set. The number of aliphatic attached chlorines exceeds the maximum occurrences of this fragment in a single compound in the training set (8 in Dechlorane Plus, maximum 6 in the training set). Therefore, the substance is not considered to be within the estimation domain of the model.

U.S. EPA (2011) reported another measured value of 2.49E-04 mg/L [240 ng/L] at 25 °C (Scharf, 1978). In EPI Suite (U.S. EPA, 2012), a measured water solubility of 4.4E-08 mg/L at 25 °C is reported citing a HPV Robust Summary as the source; this result is discounted given the discrepancy between the value quoted and the original source (4.4E-05 mg/L, Chou *et al*., 1979).

Property	Value [Unit]	Reference/source of information/remarks
Partition coefficient air/water (log value) **[log K_{AW}]**	*No data were provided by the Registrant.*	The following log K_{AW} values at 25 °C are estimated based on the Henry's Law constant: -3.2 (from measured water solubility and estimated vapour pressure) 0.44 (from measured water solubility and vapour pressure) -2.8 (from EPIWIN predicted water solubility using log K_{OW} of 9 and vapour pressure) -3.5 (from HENRYWIN v.3.20, predicted from structure using Bond Method). See discussion of Henry's Law Constant (Section 3.2.2 of Appendix 1) for further details.
Partition coefficient n-octanol/air (log value) **[log K_{OA}]**	*No data were provided by the Registrant.*	A log K_{OA} of 14.8 is estimated using KOAWIN (U.S. EPA 2012). This is a simple ratio of the octanol-water (log K_{OW} 11.3) and air-water (log K_{AW} -3.5) partition coefficients calculated within EPI Suite. There is uncertainty in this value resulting from uncertainty in the estimated K_{OW} and K_{AW} (see above). Using a log K_{OW} of 9, a log K_{OA} of 12.5 is estimated with a log K_{AW} of -3.5, or 8.6 with a log K_{AW} = 0.44.
Henry's Law Constant	*No data were provided by the Registrant.*	The following values were obtained using a range of estimation methods (including a structural fragment based QSAR method) in light of the uncertainty in vapour pressure and solubility measurements and predictions: 1.4 $Pa.m^3/mol$ at 25 °C (from measured water solubility and estimated vapour pressure) 6800 $Pa.m^3/mol$ at 25 °C (from measured water solubility and extrapolated vapour pressure) 41 $Pa.m^3/mol$ at 25 °C (from EPIWIN predicted water solubility using log K_{OW} of 9 and vapour pressure) 0.75 $Pa.m^3/mol$ at 25 °C (from HENRYWIN v.3.20, predicted from structure using Bond Method). The Bond method training set comprises much smaller molecules than Dechlorane Plus, which are generally much more soluble and of higher vapour pressure than the substance, although the predicted Henry's Law constant is mid-range for the method. It is therefore difficult to estimate the uncertainty of the predicted values. See also Section 3.2.2 of Appendix 1 for further discussion.
Solubility in organic solvent[5]	n-Octanol solubility: 470 mg/L (to the nearest 10 mg/L) at 25 °C	Reliability 1: non-guideline study conducted in a GLP facility but not formally to GLP (reference not provided, but it appears to have been conducted in the UK in 2013) Approximately 2 g sample was weighed into a 125 mL conical flask and 20 mL n-octanol was added. A magnetic stirrer was placed on a thermostatic water bath overnight followed by slow stirring. Stirring was stopped and test solutions containing insoluble test substance were allowed to settle for 30 minutes before filtration under gravity. Clear colourless filtrates were obtained and test solution was analysed using GC-ECD without further dilution. The solubility in octanol is used as part of the assessment of octanol-water partitioning and also bioaccumulation. Although the test solution was filtered, it is not known whether the reported result represents truly dissolved substance.

2 Harmonised classification and labelling

No harmonised classification is reported for Dechlorane Plus (CAS No. 13560-89-9) in Annex VI of Regulation (EC) No. 1272/2008 (CLP Regulation).

There are no proposals for new or amended harmonised classification of Dechlorane Plus (CAS No. 13560-89-9) on the Registry of Intention.

The Registrant has not proposed classification for any hazard.

The European Chemical Agency (ECHA) online Classification & Labelling (C&L) Inventory database, which was checked on 13 July 2017, reports a joint submission (consisting of 92 notifiers) indicating no classification according to the CLP criteria. In addition, 78 notifiers have classified the substance as Acute Toxicity Category 4, H332 Harmful if inhaled.

3 Environmental fate properties

3.1 Degradation

3.1.1 Abiotic degradation

3.1.1.2 Hydrolysis

The Registrant waived the hydrolysis endpoint in accordance with Column 2 of REACH Annex VIII because the substance is highly insoluble in water. Dechlorane Plus does not contain any hydrolysable groups. Hydrolysis is not expected to be a relevant fate process.

3.1.1.3 Oxidation

The Registrant considers that an estimated first-order rate constant of 1/10 000 000 000 s^{-1} (corresponding to a half-life of 2 100 years) for oxidation in water by Chou *et al*. (1979) is unreliable as the calculation method was not described.

The potential for phototransformation in air has been predicted using the AOPWIN Program (v1.92). Under standard atmospheric conditions, the half-life is predicted to be 17 hours at 25 °C, based on reaction with hydroxyl radicals in the vapour phase. Complete information on training set development for this well-established predictive method is not publicly available, although hydroxyl radical rate constants for numerous polycyclic and highly chlorinated or brominated substances are represented in the training set. The molecular weight of Dechlorane Plus is high relative to substances in the training set, so the predicted half-life may be misleading. However, given the very low vapour pressure of this substance (see Section 1.5), the relevance of this estimate is low. The AOPWIN output also estimates that the fraction sorbed to airborne particulates is between 0.95 and 1 depending on the method used, and notes that "*the sorbed*

[5] Occidental Chemical Company (2004) refers to a study from 1978 that mentions a solubility in n-octanol of 264 - 346 (average 305) ppb (µg/L) at 25 °C. No further details are available, but the result was obtained "after partitioning" (presumably with water, as the data entry is for the water solubility end point) so this is probably not a true solubility value.

Product literature (OxyChem, 2007) provides further values (all in units of g/100 g solvent at 25 °C) as follows: benzene 2.0, xylene 1.0, styrene 1.8, trichloroethylene 1.4, methyl ethyl ketone 0.7, n-butyl acetate 0.7, hexane 0.1, methyl alcohol [methanol] 0.1. The analytical information provided in the REACH registration dossier mentions that the substance is "insoluble" in methanol, but "soluble" in tetrachloroethane, dichloromethane and tetrahydrofuran.

fraction may be resistant to atmospheric oxidation."

3.1.1.3 Phototransformation/photolysis

3.1.1.3.1 Phototransformation in air

Details of available studies are summarised in Appendix 1 (Sverko *et al*., 2008; Wang *et al.*, 2011; Li *et al*., 2013b; Wang *et al.*, 2013b; Tao *et al*., 2015). These suggest that significant photodegradation can potentially occur (with the formation of at least mono-dechlorinated substances), and that the anti- isomer might be more photodegradable than the syn- isomer. However, these studies cannot be directly related to natural conditions. The light intensity might not be comparable, and the use of organic solvents could promote the generation of hydrogen radicals (especially as the substance itself does not show any significant light absorption above 260 nm).

Dechlorane Plus will be mainly adsorbed on particulates in air (see Section 3.2.1). Particulates will shield the substance from light and inhibit radical reactions.

Nevertheless, monitoring studies provide some evidence of changes in isomer ratios with increasing distance from the source (further details are provided in Section 3.1.1.3.1 in Appendix 1). For example, Möller *et al.* (2010) found that the f_{anti} value decreased with decreasing northern latitude ($r = 0.974$, $p < 0.01$) from 0.63 to around 0.33 southwards of the equator. Yang *et al*. (2012) also found that the mean f_{anti} value was highest in spring and lowest in the autumn, although the differences were not statistically significant ($p > 0.05$). These observations are often explained as being due to the effects of UV light (i.e. the anti- isomer degrading more rapidly than the syn- isomer), although Sverko *et al*. (2011) point out that it may reflect isomerisation of the anti- isomer to the syn- isomer.

Overall, atmospheric phototransformation/photolysis is likely to be of relatively low relevance to the overall fate of the substance, but may account of some of the changes in isomer fractional abundance that are observed in some matrices compared to the commercial products.

3.1.1.3.2 Phototransformation in water

Details of available studies are summarised in Appendix 1 (Chou *et al.*, 1979). In general, aquatic photolysis is unlikely to be a significant fate process in natural waters, since light is attenuated with increasing water depth and shading. Radical reactions may also be inhibited by humic substances. The available information suggests that phototransformation in water is a potential but insignificant removal process for Dechlorane Plus.

3.1.1.3.3 Phototransformation in soil

No data were reported by the Registrant. Similar to water, this is unlikely to be a significant removal pathway.

3.1.2 Biodegradation

3.1.2.1 Biodegradation in water

3.1.2.1.1 Estimated data

The aerobic biodegradation potential of the substance can be assessed using BIOWIN v4.10 (U.S. EPA, 2012). It has six different models which have been developed based on expert

judgment. The program outputs for the non-linear model (BIOWIN 2), ultimate biodegradation (BIOWIN 3) and the MITI non-linear model (BIOWIN 6) can be used as a screening assessment of persistence (P) in accordance with the REACH Guidance R.7b (ECHA, 2017b). The following results indicate that a substance may be persistent:

BIOWIN 2: Does not biodegrade fast (<0.5) or
BIOWIN 3: ≥months (< 2.25 (to 2.75)[6])
BIOWIN 6: Not readily biodegradable (<0.5) and

Inputting the structural details of Dechlorane Plus results in a BIOWIN 2 value of 0, a BIOWIN 3 value of -1.60 and a BIOWIN 6 value of 0. These values are all significantly below the cut-offs, indicating that Dechlorane Plus is not expected to be aerobically biodegradable.

With regard to validity, the BIOWIN models are based on structural group contributions and there is no defined estimation domain as such. The software providers acknowledge that multiple occurrences of a contributing positive fragment group can sometimes lead to incorrect prediction of rapid degradation. In view of the group fragment values present in Dechlorane Plus, and the prediction of "not biodegradable", this is unlikely to be a problem for the substance.

The BIOWIN model validity is considered in detail in the appendix (section 3.1.2.1.1). In summary the BIOWIN 2 and 3 model estimates have a degree of uncertainty due to (i) the lack of fragment coefficients to represent the whole Dechlorane Plus structure and, (ii) the number of identified fragments exceeding the maximum of occurrence in training set substances. The latter issue does not appear to affect the prediction that Dechlorane Plus is not biodegradable. For BIOWIN 6, the Dechlorane Plus structure is fully represented by the model fragments. The number of each fragment in the Dechlorane Plus structure is also within the range of the training set. The training set substances include two chemicals, which contain the hexchloronorbornene moiety of Dechlorane Plus. The model predictions for those substances do agree with the measured data. Overall BIOWIN 6 is considered to be a relevant and reliable model for Dechlorane Plus. The model provides support to the BIOWIN 2 and 3 predictions as all three models are consistent in predicting that Dechlorane Plus does not biodegrade fast.

It is concluded that based on the estimated data and associated uncertainties, that Dechlorane Plus is unlikely to be biodegradable.

Overall it can therefore be concluded, based on estimated data, that Dechlorane Plus is unlikely to be biodegradable.

3.1.2.1.2 Screening tests

Two old biodegradation screening studies were included in the registration dossier as key studies, and further details are provided in Appendix 1 (Boudreau, 1973; Chou *et al.*, 1979). Neither was performed according to a standard test guideline method, and neither assessed biodegradation using biological oxygen demand or carbon dioxide evolution, which would be appropriate for such an insoluble substance. No biodegradation was observed over 21 days in the first test (based on analysis of the test substance). In the second, degradation was determined as loss of radioactivity; significant loss was observed after 6 weeks' [42 days'] incubation under aerobic conditions, but no mass balance was performed, no metabolites were detected, adsorption to sludge or bacteria cannot be excluded and the position of radiolabelling in the molecule was not described. Consequently the Registrant concluded that Dechlorane Plus is non-biodegradable.

In view of the inappropriate test methods used, these screening studies provide no reliable information on the biodegradation potential of the substance.

[6] For substances fulfilling this but BIOWIN 3 indicates a value between 2.25 and 2.75 more degradation relevant information is generally warranted.

A modified MITI (OECD TG 301C) study using an activated sludge inoculum was conducted in 1974, and the limited details are summarised in Appendix 1. Dechlorane Plus achieved 0.6 % of its theoretical biochemical oxygen demand (BOD) over two weeks and 0.3 % degradation was determined by gas chromatography. The reliability of this study cannot be assessed.

3.1.2.1.3 Simulation tests (water)

No simulation data were presented by the Registrant. A test would be technically very challenging given the reported solubility in pure water.

3.1.2.2 Biodegradation in sediment

No data are available in the registration dossier.

Qiu *et al*. (2007) measured Dechlorane Plus in a sediment core from central Lake Ontario, Canada. There was a linear trend (r^2 = 0.739) of increasing f_{anti} values with time, from an average of 0.76 in surficial (recent) sediments to >0.90 in the deeper layers corresponding to around 1980. This suggests that the anti- isomer could be more persistent than the syn- isomer in sediment (although the variation of f_{anti} in commercial batches over this time period is not known, and it could also reflect the isomerisation of the syn- isomer to the anti-).

Wang *et al*. (2010) measured f_{anti} values in two freshwater sediment samples collected from a canal close to the Chinese manufacturing facility. An f_{anti} value of 0.76 in the surficial layer (0-5 cm) contrasted with a value of 0.70 in a deeper layer (15-20 cm). The study authors contrast these with the measured f_{anti} value of the Chinese commercial product (0.60) and speculate that this implies a stereoselective depletion of the syn- isomer in sediment.

Fang *et al*. (2014) investigated the distribution of Dechlorane Plus in marine sediments from South Korea. The highest concentrations (451.2 and 149.9 µg/kg dw for the two bays studied) were detected in the finest grain size (< 10 µm). The f_{anti} in the two fractionated samples increased with reduced grain size and significantly correlated with organic carbon content. The study authors hypothesised that the enrichment of the anti- isomer was likely to be due to preferential biodegradation of the syn- isomer in the sediment.

Discussion

Dechlorane Plus is very hydrophobic, so it is not surprising that these three academic studies indicate that it accumulates in sediments. The study of Qiu *et al*. (2007) suggests that it can still be present over thirty years after initial deposition. However, it is not possible to estimate sediment degradation half-lives. This is because the initial amount of substance deposited in the sediment is unknown, and the nature of the sediment (e.g. in terms of heavy metal content and presence of other substances, which may inhibit micro-organisms) is not described.

3.1.2.3 Biodegradation in soil

No standard simulation data are available. The registration dossier summarises a monitoring study by Wang *et al*. (2010) which indicates that the substance can be detected in layers up to one metre deep (see Appendix 1), but it cannot be used to estimate soil degradation half-lives for similar reasons to the sediment studies.

3.1.2.4 Analogue data

In view of the lack of definitive degradation half-life data in water, sediment and soil it is appropriate to consider information from analogues. As discussed in Section 1.4 there are ten potential analogues to Dechlorane Plus based on a common hexachlorinated norbornene moiety:

chlordane, heptachlor, dieldrin/endrin, aldrin, endosulfan, chlorendic acid, Dechlorane 602, Dechlorane 603, Dechlorane 604 and Chlordene Plus.

The closest structural analogues are Dechlorane 603 and Chlordene Plus. No standardised degradation data appear to be available for these (they are not registered under REACH and there are no data available from a search of the OECD eChemPortal[7] (accessed 13 July 2017)).

Five of the substances (chlordane, heptachlor, dieldrin/endrin, aldrin and endosulfan) are identified as Persistent Organic Pollutants (POPs) under the Stockholm Convention. The persistence of many of the early POPs was determined based on their favourable chemical properties for long-range transport and their detection in compartments and biota in the Arctic. Measured property data are presented in Appendix 1. There are few measured environmental half-lives, which is not surprising as many were banned before formal test guidelines or requirements for these existed.

Simulation test data for endosulfan indicate that at least the β isomer would be considered very persistent (vP) in some soils. In addition, the primary degradant (a sulfate) would meet the vP criterion in a number of soils. In sediment, the metabolites would be at least P (with an unbounded DT_{50} value of 120 days). None of the data for endosulfan indicate rapid breakdown of the chlorinated norbornene moiety, and very little mineralisation were observed in the studies (<5 %).

The use of the five POPs as pesticide active substances implies that they are reasonably bioavailable, which is confirmed by their high aquatic toxicity (not reported here). A comparison of the physico-chemical data of Dechlorane Plus with the POPs (summarised in Section 1.4) suggests that it is likely to be much less bioavailable as it is considerably less water soluble, and has a much higher log K_{OW} value. In addition, those POPs that do show signs of degrading have functional groups (e.g. dichlorocyclopentyl for chlordane, monochlorocyclopentenyl for heptachlor and sulfite for endosulfan) that are not present in Dechlorane Plus (which is a less polar molecule as a result). The POPs are all agreed to be environmentally persistent (within the meaning of the Stockholm Convention[8]). These provide a "best case" indication of persistence for Dechlorane Plus, i.e. it is expected to be at least as persistent as POPs with higher bioavailability and polarity.

There are no simulation data for chlorendic acid despite its relatively high water solubility (168 mg/L). Although it appears to rapidly degrade under UV light in water (half-life 5 days) and on solid surfaces (half-life 16 days), the half-life is much longer in soil (140 d at 1 mg/kg; 280 d at 10 mg/kg). No degradation was observed in a test for ready biodegradation after 31 days (based on removal of dissolved organic carbon). It therefore screens as being potentially P/vP. This is a much more polar and hydrophilic molecule than Dechlorane Plus, i.e. Dechlorane Plus is likely to degrade much more slowly than this substance.

In view of the lack of reliable environmental half-life data for most of the POPs, BIOWIN (v4.10) predictions for each of them and chlorendic acid are included in Table 7. As described in Section 3.1.2.1.1, conclusions about aerobic biodegradation potential can be drawn based on predictions from three of the models (BIOWIN 2, 3 and 6). The applicability of BIOWIN to these substances will be the same as for Dechlorane Plus (see discussion in Section 3.1.2.1.1).

Table 7: Summary of predicted and measured degradation data for Dechlorane Plus analogues[9]

Substance	CAS no.	BIOWIN prediction	Measured half-life data
Chlordane	57-74-9	BIOWIN 2 = 0	Sediment study suggests

[7] https://www.echemportal.org/echemportal/index.action

[8] That is, the half-life in water is greater than two months, or the half-life in soil is greater than six months, or the half-life in sediment is greater than six months; or the chemical is otherwise sufficiently persistent to justify its consideration within the scope of the Convention.

[9] Note that only the POP analogues and chlorendic acid are included here as these have measured (as well as predicted) degradation data available. Aldrin transforms to dieldrin.

Substance	CAS no.	BIOWIN prediction	Measured half-life data
		BIOWIN 3 = 0.27 BIOWIN 6 = 0	reduction in concentration by 70 % in 414 days
Heptachlor	76-44-8	BIOWIN 2 = 0 BIOWIN 3 = 0.53 BIOWIN 6 = 0	Rapid hydrolysis Soil DT_{50} = 9-10 months or 2 years (shorter half-lives lack supporting information)
Dieldrin	60-57-1	BIOWIN 2 = 0 BIOWIN 3 = 0.67 BIOWIN 6 = 0	Very little degradation detected in soil
Aldrin	309-00-2	BIOWIN 2 = 0 BIOWIN 3 = 0.72 BIOWIN 6 = 0	No quantitative information
Endosulfan (α and β isomer)	115-29-7	BIOWIN 2 = 0 BIOWIN 3 = 0.62 BIOWIN 6 = 0	Soil DT_{50} = 25-128 days Metabolite: soil DT_{50} = 123 - 391 days Water/sediment DT_{50} >120 days
Chlorendic acid	115-28-6	BIOWIN 2 = 0 BIOWIN 3 = 1.39 BIOWIN 6 = 0	0 % mineralisation in 31 days

The results for BIOWIN 2 and 6 are zero for all substances, indicating a similarly low potential for biodegradation as Dechlorane Plus. The results for BIOWIN 3 indicate that Dechlorane Plus (-1.60, see Section 3.1.2.1.1) may resist biodegradation more than all of the POPs. This appears to be due to the factor added for each chlorine atom in the model. While the same atom addition applies to the BIOWIN 2 and 6 models, these structure-activity relationships appear to only return zero or positive values. As a minimum, Dechlorane Plus is not predicted to be more rapidly biodegraded than the analogues. Chlorendic acid appears to provide a "best-case", as it is the most water soluble of the group, yet it fails to undergo ready biodegradation.

Fragment considerations

Dechlorane Plus differs from all of the analogues by virtue of its cyclooctane ring which links the two chlorinated norbornene fragments. If this ring could be opened by biotic or abiotic degradation, chlorendic acid (see above) could be formed as a potentially persistent metabolite. Whilst likely to be persistent, the negative log K_{OW} of chlorendic acid suggests that it is not bioaccumulative. Cyclooctane (CAS no. 292-64-8) is not currently registered under REACH, and a literature search[10] has not located any degradation data for the substance.

Cyclooctane is a relatively simple alkane and predicted to be of moderate water solubility and log K_{OW}. EPIWEB v4.1[11] predicts that it can be rapidly degraded. but in the absence of measured biodegradation data, it is not possible to confirm this. The absence of the CH2 (cyclic) fragment in the BIOWIN 2 and 3 models, means that the prediction is based on molecular weight (see also the discussion of this issue for Dechlorane Plus in Section 3.1.2.11). Furthermore, comparison of measured and predicted biodegradation data for other cycloalkanes indicates that caution should be exercised when only a prediction is available. For example, while measured and predicted data indicate that cyclohexane is readily biodegradable (EC, 2004), measured data for methylcyclohexane shows that it is not ultimately biodegradable contrary to the BIOWIN prediction (ECHA, 2017d). In addition, the two chlorinated norbornene rings in Dechlorane Plus constrain the flexibility of the cycloalkane structure (there are two structural isomers), which may also affect degradation potential. Hoh *et al.* (2006) suggested that because of the

[10] Including the draft voluntary assessment of gasoline under the Existing Substances Regulation, and recent assessments submitted to the OECD.

[11] Relevant predicted results for cyclooctane, C1CCCCCCC1, are: log Kow = 4.16, water solubility = 4.9 mg/L and 14.1 mg/L using WSKOW and WATERNT respectively. BIOWIN predictions: BIOWIN2 = 0.80 (Biodegrades Fast); BIOWIN3 = 2.95(weeks); BIOWIN6 = 0.76 (Biodegrades Fast).

configuration of the pendant chlorocyclopentene moieties, the anti- isomer would be more susceptible to biological attack than the syn- isomer.

Biodegradation pathway considerations

The EAWAG-BBD Pathway Prediction tool[12] for biodegradation can be used to explore the possible aerobic degradation pathways for Dechlorane Plus. The model suggests two possible pathways: hydroxylation of a secondary carbon (on the cyclooctane ring) and hydroxylation of a tertiary carbon (the carbon atom common to the cyclooctane and norbornene rings)[13]. Both pathways are predicted to be of neutral likelihood, which is an assessment of the general pathway rather than specific to the substance. As noted in Section 1.3, dechlorinated substances have been detected in the environment. These may be reductive transformation products, but might also be impurities in the commercial substance. None of the substances are predicted to be formed using this tool.

Similar to Dechlorane Plus, prediction for cyclooctane also suggests degradation via hydroxylation of a secondary carbon on the ring. Both cyclooctane attached to a single norbornene ring, and chlordane (the only analogue that contains a secondary carbon, in a pentyl ring) are predicted to degrade via both the tertiary or secondary carbon. Heptachlor and chlorendic acid are indicated to have the same tertiary carbon biodegradation pathways as Dechlorane Plus.

The prediction for endosulfan suggests that conversion to the sulfate is favoured over the hydroxylation of the tertiary carbon which agrees with the experimental observation described in the POPs dossier. The epoxidation of dieldrin is favoured over the hydroxylation of the tertiary carbon.

The predictions suggest that the pathways available for Dechlorane Plus are no different to those for the analogues. However, the tool does not provide a prediction of rates for any part of the pathway, and it is not possible to determine how degradation rates will vary between the chemicals. The two degradation pathways predicted to exist for Dechlorane Plus do not appear to be rapid based on the known persistence of the analogues where these pathways also exist (e.g. dieldrin). For dieldrin one pathway is suggested to be more likely than the other. However, the persistence of the substance suggests that both are slow.

As a further indication of the limited biotransformation potential of Dechlorane Plus, Tomy *et al*. (2008) were unable to identify any Dechlorane Plus metabolites in a 161-day dietary exposure study with juvenile Rainbow Trout (*Oncorhynchus mykiss*) (see Section 3.4.1.2.2).

3.1.3 Other data

A study investigating changes in sewage sludge concentrations is briefly summarised in Appendix 1 (de la Torre *et al*., 2011), but does not provide any information to enable an environmental half-life to be estimated.

3.1.4 Summary and discussion of degradation

No degradation studies are available that meet modern regulatory standards. Key studies cited in the registration dossier cannot be considered reliable. However, it is possible to conclude that Dechlorane Plus is unlikely to undergo significant abiotic degradation. There are no environmental simulation studies, but evidence from quantitative structure-activity relationships (QSARs), analogue read-across and biodegradation pathway considerations all indicate that the

[12] http://eawag-bbd.ethz.ch/predict/

[13] Secondary aliphatic → secondary alcohol (bt0242), tertiary aliphatic → tertiary alcohol (bt0241).

rate of biodegradation is likely to be very slow in the environment. In particular, BIOWIN predictions suggest that Dechlorane Plus has a biodegradation potential similar to – or possibly lower than – analogues that are POPs. The much more water soluble chlorendic acid provides the "best case" analogue in terms of persistence, and even this is not readily biodegradable. Two potential biodegradation pathways are predicted for Dechlorane Plus. One of these is common to all of the POP analogues, and both are common to chlordane. Neither of the pathways is predicted to be "likely" or "very likely", suggesting they are not favourable ways for the molecule to degrade. This agrees with sediment degradation data for chlordane, which suggest slow degradation. Overall, the non-test information provides a strong signal that Dechlorane Plus will degrade slowly and so have a long half-life in sediments and/or soils.

Field studies (e.g. Qiu *et al*., 2007) provide some limited evidence of high persistence (in that Dechlorane Plus was found in a sediment core layer corresponding to around 1980). A fish bioaccumulation study (Tomy *et al*., 2008) also suggests a limited potential for rapid biotransformation.

The same limited field data provides the only information for the fate of the two isomers of Dechlorane Plus. At present there is no strong evidence to contradict the assumption that both isomers have a similar level of persistence based on the conclusion drawn for the registered substance.

3.2 Environmental distribution

3.2.1 Adsorption/desorption

No reliable information on the organic carbon-water partition coefficient (K_{OC}) is included in the registration dossier, but a non-standard study investigating adsorption to sediment is summarised in Appendix 1 (Chou *et al.*, 1979).

In view of the fact that Dechlorane Plus is a highly insoluble substance with a high log K_{OW} and (relatively) high solubility in n-octanol, it is expected to have a high potential for adsorption. KOCWIN v2.00 (U.S. EPA, 2012) can be used to predict log K_{OC} values of 7.7 (Molecular Connectivity Index estimation method) and ≥7.8 (log K_{OW}-based estimation method; using the log K_{OW} value ≥9). The substance is within the domain of the method because the molecular weight is within the molecular weight range of the training set, and no fragment corrections are applied. The Registrant assumes a log K_{OC} of 8 in their Chemical Safety Report (CSR).

These predicted values indicate a high adsorption potential for Dechlorane Plus, suggesting that sediment and soil are more relevant environmental compartments than water (i.e. they are likely to be major sinks).

3.2.2 Volatilisation

No information on volatilisation was reported in the registration dossier.

The volatilisation potential of the substance from water can be estimated based on the available vapour pressure and water solubility data for the substance, and also by reference to QSAR-estimated values (see Appendix 1). Whilst there is some uncertainty in the values of water solubility and vapour pressure (see Section 1.5), the calculated HLC based on measured input data is $\geq 1.39 \times 10^5$ Pa.m^3/mol at 25 °C, suggesting that Dechlorane Plus could be volatilised from water. However, strong adsorption to organic matter is likely to make this fate pathway less important in natural waters.

Given the very low vapour pressure and high K_{OW} of the substance (i.e. a high K_{OA}), Dechlorane Plus will be mainly adsorbed on particulates in air. This has been demonstrated by monitoring studies, with mean fractions on particulates of 97 % or more (e.g. Hoh *et al*., 2006; Ren *et al*.,

2008; Wang *et al*., 2010), although Möller *et al.* (2010) measured it as around 80 ± 30 % and Yang *et al*. (2012) only detected Dechlorane Plus in the particulate phase.

3.2.3 Distribution modelling

No information on distribution modelling was reported in the registration dossier.

The CEMC Level III Fugacity Model v 2.80 (CEMC, 2004) can be used to model the distribution of Dechlorane Plus. The physico-chemical property values used in the model are those selected in Table 7: water solubility 2.0×10^{-6} mg/L; vapour pressure 4.6×10^{-4} Pa; log K_{OW} 9. The degradation half-lives used in the model environment are: air 16.8 hours; water 1.8×10^{4} hours (assuming photodegradation) or 8.4×10^{6} hours (assuming no photodegradation); soil 8.4×10^{6} hours.

If Dechlorane Plus is assumed to be released at equal rates to air, water and soil, the model predicts the following distribution: air 3.7×10^{-3} %, water 0.087 %, soil 96.5 % and sediment 3.45 % (the two half-lives in water give the same result). The substance has a very low vapour pressure. If it is released only to water (with no application to soil, including WWTP sludge), the calculated distribution is very different: air 9.0×10^{-5} %, water 2.46 %, soil 1.9×10^{-3} % and sediment 97.5 %. It should be noted that there is uncertainty in the property values used in the modelling and hence uncertainty in the results. More than 97 % of the Dechlorane Plus in the atmosphere is likely to reside in the particulate phase (see Section 3.2.1).

Using the OECD P_{ov} and LRTP Screening Tool v2.2 (Wegmann *et al*., 2009), the results obtained for Dechlorane Plus (see Section 3.3) suggest that it has a relatively low transfer efficiency from air to surface media[14] of 3×10^{-4} %.

Sverko *et al*. (2011) studied air-water exchange using the data measured by Möller *et al*. (2010) in the marine environment. The mean concentrations in air (gas phase) and seawater (dissolved phase) were 0.12 pg/m^3 and 0.009 pg/L, respectively, in the East Greenland Sea and 0.028 pg/m^3 and 0.044 pg/L, respectively, along the Atlantic transect. The resulting fugacity fraction[15] is near unity, suggesting net gaseous deposition of Dechlorane Plus to seawater.

Sverko *et al*. (2011) also compared the ratio of concentrations in air and soil reported by Wang *et al*. (2010) with an estimate based on the log K_{OA} value. The good agreement suggested that Dechlorane Plus in the gas phase originated from soil volatilization. However, they did not perform a similar analysis to compare gas phase with particulate concentrations.

3.3 Data indicating potential for long-range transport

No information on long range transport potential was reported by the Registrant. However, a summary of Xian *et al*. (2011) given as supporting monitoring information in the IUCLID file states that "long-range atmospheric transportation of Dechlorane Plus has been observed in

[14] Transfer efficiency in this model is defined as "the ratio of the deposition mass flux from air to surface media in a region adjacent to the region to which the chemical is released and the mass flux of the chemical emitted to air in the release region"

[15] This is used to assess equilibrium status of a chemical between two interacting phases, in this case air and water:

Fugacity fraction = C_A / (C_A + $K_{AW}C_W$)

where C_A is the air concentration (in pg/m^3), C_W is the water concentration (in pg/L)

Values equal to 0.5 indicate air–water equilibrium and no net gas exchange. Values < 0.5 indicate net volatilization from water, and values > 0.5 indicate net gaseous deposition to water.

remote regions, indicating a global presence of Dechlorane Plus."

The potential for long range transport (LRTP) can be modelled using the OECD P_{ov} and LRTP Screening Tool (Wegmann *et al*., 2009). Table 8 shows a summary of input data for Dechlorane Plus and context for the model. Uncertainties in the values of vapour pressure, water solubility, log K_{AW} and log K_{OW} for Dechlorane Plus (see Sections 1.5 and 3.1.1.3.1) all affect the input parameters for the calculation.

Table 8: Input properties used in assessing long range transport potential

Input property	**Value**	**Context in terms of property input data for reference POPs in the model**
Log K_{AW}	3.5 – 0.44	Within the range
Log K_{OW}	9	Above the upper limit (i.e. relatively lipophilic)
Half-life in air	16.8 h	Within range, relatively short half-life
Half-life in water	Variable inputs used to explore range: 8.4×10^6 h (assuming no photodegradation); 1.8×10^4 h (assuming photodegradation)	Relatively persistent (unless photodegradation assumed)
Half-life in soil	8.4×10^6 h	Relatively persistent

Whilst no absolute criteria for classifying chemicals as compounds with high or low overall persistence (P_{ov}) and LRTP have been established, the threshold values established by Klasmeier *et al*. (2006), based on limit values for reference POPs, can be applied (P_{ov} = 195 days, Characteristic Travel Distance = 5 097 km, and Transfer Efficiency = 2.248 %):

- If no degradation in water or soil is assumed for Dechlorane Plus, the potential for long range transport varies significantly depending on the log K_{AW} value used, with a characteristic travel distance of between 350 and 2,239km. The overall half-life (P_{ov}) for Dechlorane Plus is predicted to be very long, at between 16 565 and 501 448 days, and it is predicted to have a transfer efficiency from air to surface media of between 0.00193 and 7.63%. In this model, the fraction of Dechlorane Plus in air present in aerosols varies between 0.03 and 96.4 %.

- Taking photodegradation as a potentially relevant degradation pathway into account has little impact as the overall half-life (P_{ov}) for Dechlorane Plus remains very high, at between 16 411 and 343 days. The characteristic travel distance and transfer efficiency are unchanged.

It can be seen that the results very much depend on the choice of log K_{AW} value, which has a range across four orders of magnitude depending on the values used to calculate it.

Although there are significant uncertainties in the physico-chemical data set, the predicted range of log K_{OA} (>7.25 to >12) and log K_{AW} (3.5 to 0.44) values for Dechlorane Plus also suggest a low potential to reach the Arctic according to the criteria cited by Wania (2006) and Brown and Wania (2008).

The results from this modelling are uncertain, largely because most of the input parameters are estimated. In the context of the input properties for reference chemicals in the OECD model, the properties of Dechlorane Plus show that it has comparable or higher hydrophobicity than reference POPs, is generally of comparable volatility (based on log K_{OA} and log K_{AW} values). Atmospheric photodegradation is possible but adsorption to particles is likely to significantly extend the half-life (see also Section 3.1.1.3.1). Modelling may be of limited value for substances that have low vapour pressure and adsorb strongly to particulates in the air. Atmospheric transport for such substances is likely to be governed by the fate of these particulates. This can result in long-range transport to remote regions when atmospheric conditions permit (e.g. during

dry periods). This is confirmed for Dechlorane Plus by monitoring evidence: Appendix 1 summarises several studies that demonstrate detection in the Polar regions as well as high mountains (Kaj *et al*., 2010; Möller *et al*., 2010; Sverko *et al*., 2010a [ABST]; Möller *et al*., 2011; Möller *et al*., 2012; Xiao *et al*., 2012; Arinaitwe *et al*., 2014; Newton *et al*., 2014; Salamova *et al*., 2014; Vorkamp *et al*., 2014; Ma *et al*., 2015; Na *et al.,* 2015; Vorkamp *et al*., 2015), and also a study by Okonski *et al*. (2014) that investigated the presence of Dechlorane Plus on different sizes of particulate. Several of the studies measured Dechlorane Plus in seawater (Möller *et al*., 2010; Möller *et al*., 2011; Möller *et al*., 2012). The authors of these papers suggest that this reflects either riverine emission or atmospheric deposition (rather than long range transport via particles in water).

In summary, although the modelling data give mixed results, possibly as a consequence of the physicochemical characteristics of the substance, the monitoring data indicate that Dechlorane Plus can undergo long range transport, most probably as a result of adsorption to particulates in the atmosphere.

3.4 Bioaccumulation

3.4.1 Bioaccumulation in aquatic organisms (pelagic & sediment organisms)

3.4.1.1 Screening information

The log K_{OW} of Dechlorane Plus is estimated to be ≥9 (see Section 1.5). It therefore screens as potentially very bioaccumulative (vB). The log K_{OA} above 5 together with high log K_{OW} also suggest it may have a high bioaccumulation potential in air-breathing wildlife.

The bioconcentration factor (BCF) of an organic chemical can often be predicted using QSAR correlations with log K_{OW}, especially if the substance is not metabolised very readily. For the purposes of this assessment, some standard methods have been used to predict the following fish BCF values assuming a log K_{OW} of 9 as the lower limit (further details are provided in Appendix 1; this also includes published predictions by Chou *et al.* (1979) which appear unreliable):

i) Non-linear equation from REACH Guidance R.7c (ECHA, 2017c): ≤5 500 L/kg (unreliable)
ii) log K_{OW} >7 regression equation from BCFBAF v3.01 (U.S. EPA, 2012): ≤1 400 L/kg
iii) Arnot and Gobas (2003) method in BCFBAF v3.01: 1 200 L/kg (lower trophic level); bioaccumulation factor (BAF) 7.5 × 10^5 L/kg wet weight (ww)[16]

Given the uncertainty in the actual log K_{OW} value, all of these predictions should be considered with caution. In addition, the QSARs themselves are based on assumptions and in some cases very small data sets (see Appendix 1 for further comment). It is also relevant to note that the BCFBAF program estimates the BCF (and BAF) on the basis of the total concentration in water rather than the dissolved concentration in water (EA, 2013). The freely dissolved concentration of Dechlorane Plus would be significantly lower than the total concentration and hence the actual BCF based on dissolved concentrations may be higher than predicted above.

The REACH guidance (ECHA, 2017a) suggests several chemical properties that may limit the absorption and distribution of a chemical via passive transport. In summary, the fish BCF is unlikely to be above 2 000 L/kg if a substance has:

[16] Sverko *et al*. (2011) included estimated biotransformation rate constants with this model to derive BAFs of 5.9 × 10^4 L/kg for the anti- isomer and 1.1 × 10^5 L/kg for the syn- isomer, based on total water concentrations. BAFs at the middle and upper trophic levels were higher.

- an average maximum diameter greater than 17 Å (1.7 nm)[17], plus a molecular weight greater than 1 100 g/mol; or
- a measured octanol solubility (in mg/L) below 0.002 times the molecular weight, as an indicator of lipid solubility (in the case of Dechlorane Plus, this would be 1.3 mg/L) (without observed toxicity or other indicators of bioaccumulation).

The molecular weight of Dechlorane Plus is 654 g/mol. Using the OECD QSAR Toolbox v2.1, the maximum molecular diameter can be estimated as 1.43 nm (the minimum and effective diameters are 0.85 and 0.9 nm, respectively). Using the BCF_{max} model with mitigating factors (Dimitrov *et al*., 2005), Environment Canada estimated a maximum diameter of 1.35 – 1.48 nm (pers. comm.). Fang *et al*. (2014) performed molecular characterization of both isomers using Gaussian 03 (Revision C.02) software, showing that the syn- isomer has a higher dipole moment, slightly larger Van der Waals volume, but smaller maximal cross-sectional diameter than the anti- isomer (1.24 nm compared to 1.42 nm).[18] On the basis of these estimates, the first criterion is not fulfilled.

The registration dossier reports the n-octanol solubility of Dechlorane Plus to be 470 mg/L at 25 °C. It therefore appears that neither of the two criteria are fulfilled (i.e. the molecular size and n-octanol solubilities do not suggest that the substance is incapable of accumulation). An n-octanol solubility of around 0.5 g/L suggests that lipid solubility might be relatively high.

Fang *et al*. (2014) performed experiments using activated charcarbon to adsorb Dechlorane Plus dissolved in methanol. The adsorption results revealed that the syn- isomer was preferentially adsorbed, suggesting that it is more hydrophobic than the anti- isomer. Tomy *et al*. (2008) used a reverse-phase C18 liquid chromatography column as a surrogate for lipid, where the retentiveness of chemicals on the column is driven by the intrinsic polarity of the compound. Under isocratic conditions, the anti- isomer eluted before the syn- isomer (retention time 28.5 and 31.1 minutes, respectively) indicating that the syn- isomer is more lipophilic. These observations are consistent with measured bioaccumulation data and strongly suggests that polarity is an important factor for the differential bioaccumulation of the two isomers.

3.4.1.2 Laboratory studies

3.4.1.2.1 Aqueous exposure

The combined solubility of both Dechlorane Plus geometric isomers in pure water is below 2 ng/L (0.002 µg/L) at 20 °C (see Section 1.5). It is a very hydrophobic substance, with a presumed log K_{OW} value of ≥9. These properties make aqueous laboratory studies very difficult to carry out reliably owing to the potential difficulties in maintaining stable exposure levels at such low concentrations.

Five fish bioconcentration studies are available in the registration dossier and grey literature, which are summarised in detail in Appendix 1 (Boudreau & Rausina, 1973; Gara & Rawisina, 1975; Chou *et al*., 1979; Zitko, 1980; U.S. EPA, 2011 (citing an unpublished Japanese study)). All were carried out at concentrations significantly above the solubility limit in pure water, and only one followed a regulatory test guideline (this study is forty years old, key data are missing, and it did not achieve steady state). None of these studies is reliable. However, the longer duration studies do indicate that the substance can be taken up into fish tissues. If it is assumed that the fish were exposed to the substance at its water solubility limit (<2 ng/L), tentative BCFs

[17] A maximum molecular length greater than 43 Å (4.3 nm) is also mentioned, but EA (2009c) found that the basis for this value was highly dubious so it is not considered further here.

[18] Tomy *et al*. (2008) performed molecular modelling for the global minimum energy conformer of the Dechlorane Plus structure using semi-empirical AM1 calculations in the Spartan ES program. However, as the modelling resulted in the chair conformation for the cyclooctane moiety for both isomers, the calculations may have been unreliable, and so the values for molecular volume and dipole moments are not reported.

are estimated to be well above 10 000 L/kg, although the likely oral exposure of the fish to particulates means that these values might be a misleading indication of bioaccumulation potential. The maximum fish concentrations measured in the aqueous BCF studies were 0.385 – 8.72 mg/kg after 30 days for *Lepomis macrochirus* (Gara & Rawisina, 1975; Boudreau & Rausina, 1973) and 0.327 mg/kg after 56 days for *Cyprinus carpio* (Japanese BCF study cited by U.S. EPA, 2011). The wide range for *L. macrochirus* could be due to experimental variation (especially as the exposure conditions were variable) or could perhaps be linked to differences in lipid content and age of the test fish. It is not known if these measurements would have included any substance adsorbed to the skin or present in the gut. As steady state had not been reached it is possible that the concentrations could become higher with longer exposures.

A sixth study is summarised in Appendix 1, suggesting a BCF for plants (Sea Lettuce *Ulva pertusa*) below 100 L/kg (Zhao *et al.*, 2014). There are a number of uncertainties with this study that mean the results should be treated with caution, including variable exposure concentrations (significantly exceeding the reported water solubility limit), presence of Dechlorane Plus in controls, potential growth inhibition during the test (which may or may not be related to the test substance), and issues around the way that growth was calculated and kinetics fitted.

In summary, aqueous exposure is expected to be of limited importance in terms of bioaccumulation potential, and whilst significant uptake has been shown to occur in fish, there are no reliable measured fish BCF data.

3.4.1.2.2 Dietary exposure

OECD TG 305 advises that dietary exposure is more suitable than aqueous exposure for substances with log K_{OW} >6. Five dietary studies for fish are available and four are summarised in Appendix 1 (Zitko, 1980; Xiao *et al*., 2013; Zeng *et al*., 2014a; Hang et al., 2013 [ABST]). The **key study** is Tomy *et al*. (2008), which is summarised in full here.

Tomy *et al*. (2008) report a dietary bioaccumulation laboratory test using juvenile Rainbow Trout (*Oncorhynchus mykiss*). The fish were exposed to doses of syn- and anti- isomers of Dechlorane Plus via their diet for 49 days (uptake phase), followed by 112 days of untreated food (depuration phase) to examine bioaccumulation parameters and possible metabolic products. Each treatment group consisted of 60 fish held in 200 L aquaria. Two groups of fish (initial mean weight 50 ± 5 g) were exposed separately to food dosed with 0.79 ± 0.03 µg/g (lipid weight) of syn- isomer and 1.17 ± 0.12 µg/g (lipid weight) of anti- isomer, while a third control group was fed unfortified food. Commercial fish food was blended for 20 minutes with corn oil spiked with known amounts of each isomer, followed by a further 20 minutes stirring with an aqueous gelatin binder. The food was air-dried for 40 minutes then extruded through a 4-mm-diameter noodler, dried at 10 °C for 48 hours, and crushed into pellets that were stored in the dark at -4 °C to limit the possibility of light-induced degradation. The concentrations of both Dechlorane Plus isomers did not decline in the food from the start of the exposure to the end of the depuration. Small amounts of the syn- isomer were detectable in the unfortified food (1.5 ng/g). The average lipid content of the food was 14.3 ± 0.3 per cent. Feed was presented by sprinkling at the surface of the water and was generally consumed by each group of fish within one minute. The daily feeding rate was 1.0 % of the mean weight of fish, adjusted after each sampling period based on the mean weight of the subsample of fish that were sacrificed. Flow-through conditions were used. The influent water was at 12 °C, pH 7.9-9.1 and dissolved oxygen was always at the level of saturation. A 12 h light:12 h dark photoperiod was maintained throughout the experiment.

Four fish were sampled from each tank on days 0, 7, 14, 21, 35, and 49 of the uptake period and on days 7, 22, 35, 49, 70, and 112 days of the depuration period. Fish were always sampled 24 hours after the previous feeding. Samples were homogenized and weighed prior to accelerated solvent extraction and purification (carcass only) by Florisil chromatography. Analysis was by both high and low resolution gas chromatography-mass spectrometry analysis (GC-MS) and also liquid chromatography-UV analysis. Quality control procedures included injections of hexane after every five samples and method (procedural) blanks. Method detection limits were estimated to be 0.02 pg/g (0.6 fmoles) for both isomers. When detected,

concentrations of the Dechlorane Plus isomers in tissue of control fish were subtracted from that in fish exposed to treated food. Concentrations in fish were also corrected for lipid percent and recovery corrected using BDE-77, -126, -197, and -207.

Dechlorane Plus had no effect on growth rate, liver somatic index or mortality at the concentrations used.

Fish lipid content on days 49 and 161 were 6.9 ± 0.5 % and 7.3 ± 0.6 % for the anti- isomer treatment, 7.5 ± 0.3 % and 7.3 ± 0.7 % for the syn- isomer treatment and 8.6 ± 0.4 % and 7.1 ± 0.7 % for the untreated control group (which could indicate some variation in feeding rate in the controls), respectively (average of four fish per sample point). The growth rates of the fish were stated to be 0.0308±0.0020, 0.0316±0.0018 and 0.0353±0.0022 mass/d for the anti- isomer, syn- isomer and control fish treatments, respectively (based on a fitted linear growth model; see later discussion).

After the 49-day exposure period, neither isomer had reached a steady-state concentration in the fish. The data in the paper are shown graphically in terms of the amount of substance per fish rather than the more normal concentration units. The maximum arithmetic mean fish whole body (minus liver) mass of the syn- isomer (control- and lipid- corrected) present at the end of the uptake period was 2.2 nmole, or 1.44 µg (value read from a graph). The mass of the anti- isomer was 1.7 nmole, or 1.11 µg (value read from a graph). The presentation of data in this way is unusual; it would normally be expressed in terms of a wet weight concentration. As the lipid correction method is unclear, no attempt has been made to estimate what the concentration in fish would be at the end of the uptake phase (see also below).

The syn- isomer accumulated in the fish linearly with time (whole body minus liver) during the exposure phase with a calculated uptake rate of 0.045 ± 0.005 nmol/d. This rate corresponds to the initial slope of the plot of the amount of substance in fish against time. A similar uptake rate was also observed for this isomer in the liver. The uptake rate for the anti- isomer was calculated to be 0.018 ± 0.002 nmol/d over days 7 to 49, as there was a large increase in the amount of this isomer accumulated during the first seven days, after which time the uptake was linear. This was statistically different to the syn- isomer at the 95 % confidence level. These rates of uptake are considered further later.

The elimination of both isomers from the fish (whole minus liver) followed first order depuration kinetics according to the paper, with reported rate constants of 0.013 ± 0.003 d^{-1} (syn- isomer)[19] and 0.023 ± 0.004 d^{-1} (anti- isomer), reported assimilation efficiencies of 6.0 % (syn- isomer) and 3.9 % (anti- isomer) and calculated half-lives of 53.3 ± 13.1 days (syn- isomer) and 30.4 ± 5.7 days (anti- isomer).[20] However, Figure 1 in the paper shows that following an initial steep decrease in concentration of the syn- isomer over the first ten days of depuration, the concentration increased by a factor of two between days 59 and 100, after which the concentration declined once more. The paper does not discuss this pattern specifically. This is considered below.

Liver-specific uptake kinetics were similar to those observed in the carcass suggesting that uptake rates of the isomers are not tissue-specific. Depuration from the liver did not follow first

[19] Table 2 in the paper reports a depuration rate constant with units of "/d $\times 10^{-2}$" in the header, whereas the main text uses the unit of "/d". If the value inferred from Table 2 for the *syn*- isomer were 0.00013 d^{-1}, the half-life would be about 5,300 days. Arnot & Quinn (2015) (supporting information) show that "$\times 10^{-2}$" is an error.

[20] Sverko *et al*. (2011) applied the method to estimate *in vivo* biotransformation rates in fish developed by Arnot *et al*. (2008) to the dietary test data reported by Tomy *et al*. (2008). The estimated biotransformation rate constants (normalized to a 10 g fish at 15 ºC) were 0.015 d^{-1} and 0.0083 d^{-1} for the anti- and syn- isomer, respectively. An uncertainty analysis indicated that 95 % of the expected values are approximately within an order of magnitude of the estimates. The equivalent biotransformation half-life is 47 and 84 days for the anti- and syn- isomer, respectively. Such biotransformation half-lives are slow, and comparable to biotransformation half-lives associated with chemicals that are known to biomagnify and bioaccumulate in aquatic food webs (Arnot *et al*., 2009).

order kinetics for either isomer. Elimination of the isomers from the liver was difficult to interpret because of suspected enterohepatic circulation and redistribution of the isomers in the liver during clearance from other tissues.

The biomagnification factor (BMF) values were reported to be 5.2 for the syn- isomer and 1.9 for the anti- isomer (whole fish minus liver), suggesting that the syn- isomer is more bioaccumulative. However, it is not clear how these values were obtained and they appear too high when compared to methods of calculation more in line with the methods recommended in the OECD 305 test guideline (see further discussion below).

None of the possible metabolic products of Dechlorane Plus (including dechlorinated, hydroxylated, methoxylated and methyl sulfone derivatives) were detected in the liver.

Discussion

Dietary studies provide a more environmentally realistic exposure route for very hydrophobic substances than aqueous exposure studies, and also provide a means to dose fish to see if uptake can occur and to follow depuration kinetics. Nevertheless, studies with other highly hydrophobic substances such as decabromodiphenyl ether (ECHA, 2012a) suggest that the presence of undissolved microcrystals can affect the degree of absorption across the gut and the nature of the food may also have an influence.

The Registrant describes the Tomy *et al*. (2008) study as reliable with restrictions. Based on the information available in the paper, the study method is broadly in accordance with the OECD TG 305-III (Dietary Exposure Bioaccumulation Fish Test). Minor deviations include the use of slightly fewer fish (e.g. five fish per sampling point is recommended) and lack of information on spiked feed homogeneity and total organic carbon content of the test water. The most important missing information is the actual wet weight fish concentrations at each time point (this is not provided in the supporting information either).

The BMF calculation in the paper was based on a method described in a previous paper by the same authors (Tomy *et al*., 2004a). There are a number of differences between this method and that recommended in the OECD TG 305-III. In particular, Tomy *et al*. (2004a) report rates for certain processes rather than the equivalent rate constant that is required for calculations by the OECD TG 305 methods[21]:

- Growth rate was calculated by fitting fish and liver weight data to a linear growth model, whereas the OECD TG recommends that a growth rate constant is obtained from the slope of a plot of natural logarithm of fish wet weights against time, separately for the uptake and depuration phases. This cannot be done precisely in the absence of the raw data. However, if it is assumed that the initial fish weight was 50 g, and that the growth rate (rate of change of fish weight with time) was fitted using an equation of the form (1 + b × time), the expected fish concentrations at each time point during the study can be estimated approximately using the growth rates given in the paper (0.0308 mass/day for anti-DP and 0.0316 mass/day for syn-DP). Using these back-calculated fish weights it is then possible to estimate the rate constant for growth dilution from the slopes of plots of ln [back-calculated fish weight] versus time. Using this approach the rate constant for growth dilution (k_g) can be tentatively estimated to be around 0.01 d^{-1} for both anti-DP and syn-

[21] It is important to distinguish clearly between rates (or more correctly the rate of change) of a process and the rate constant for a process. For example, OECD TG 305 assumes that the rates of uptake, depuration and growth dilution are all first order processes, i.e. the rate of change in concentration is a result of a constant (rate constant) multiplied by the appropriate concentration. Thus, as the accumulation proceeds, the rate of the uptake and depuration changes but the rate constant remains constant. In several places Tomy *et al*. (2008) reported the rate rather than the rate constant. Typically, the rate for the initial phases of a first order process can be approximated to the initial slope of the concentration-versus-time curve. Thus it is possible to estimate approximately the required rate constant from the rate data presented in the Tomy *et al*. (2008) paper in some instances.

DP, although it should be noted that the plots show a slight deviation from linearity.

- Fish concentrations were lipid-corrected and corrected for growth dilution by multiplying them by a factor of (1 + b × time), where b is the growth rate. The method used for lipid correction is not clear, but it is possible that the data have been corrected to give them on a lipid weight basis prior to correcting for growth (although this cannot be ascertained). The OECD TG recommends that correction for growth is done separately. However, the method used by Tomy *et al*. (2008) effectively estimates the amount of substance present in the fish (rather than the concentration). Such data can be used directly to estimate the growth-corrected depuration rate constant from the slope of a plot of ln [amount per fish] versus time. This is the "alternative" method for estimating the growth corrected depuration rate constant that is discussed in the OECD TG 305. Unfortunately the raw concentration/amount data are not provided in the Tomy *et al*. (2008) paper and are only presented graphically. The amounts per fish during the depuration phase for both anti-DP and syn-DP from can be estimated the plots in the original paper and estimated the approximate value for the growth-corrected depuration rate constant (k_{2g}) to be 0.019 d^{-1} for anti-DP and 0.012 d^{-1} for syn-DP. These are reasonably consistent with the depuration rate constants of 0.023 d^{-1} for anti-DP and 0.013 d^{-1} for syn-DP reported by Tomy *et al*. (2008).

- It is also possible to estimate the overall depuration rate constant, and hence growth-corrected depuration rate constant, using the back-calculated fish weight data estimated above to convert the data on the amount per fish to a concentration in fish (µg/g (lipid?)[22]). The overall depuration rate constant (k_2) can be obtained from plots of ln [concentration (µg/g (lipid?))] versus time. Such plots have been constructed for the purposes of this assessment using the amounts per fish read from the graphs in the Tomy *et al*. (2008) paper and these give estimates for the k_2 values as 0.027 d^{-1} for anti-DP and 0.020 d^{-1} for syn-DP. Using the k_g value estimated above of 0.01 d^{-1}, results in growth-corrected depuration rate constants (k_{2g}) of approximately 0.017 d^{-1} for anti-DP and 0.010 d^{-1} for syn-DP. Given the assumptions made in estimating these values, agreement with the values of k_{2g} determined above using the amounts per fish data directly is good.

- It is also important to note that the above depuration rate constants are obtained by including the value for day 49 of uptake in the depuration plot. As noted earlier, for both anti-DP and syn-DP there was a relatively rapid decrease from the value on day 49 of uptake to that on day 7 of depuration. The inclusion of the day 49 of uptake value for the estimation of the depuration rate constant could be questioned as the fish were still being exposed on day 49. When the day 49 of uptake value is omitted, the growth-corrected depuration rate constant (k_{2g}) obtained from the amount per fish data is lower than the above values, around 0.017 d^{-1} for anti-DP and 0.0099 d^{-1} for syn-DP. Again these values are reasonably consistent with the above estimates.

- The assimilation efficiency was calculated with the equation:

$$\frac{\text{(control-corrected concentration in fish} \times \text{mass of fish)}}{\text{(control-corrected concentration in food} \times \text{mass of food eaten)}}$$

  In contrast, the OECD TG recommends that it is calculated using the equation:

  (derived concentration in fish at time zero of the depuration phase × overall (not growth-corrected) depuration rate constant) / (food ingestion rate

[22] As noted earlier, it is not clear how the concentrations have been lipid-corrected in this study.

constant × concentration in food × $(1 - e^{-(\text{overall depuration constant} \times \text{uptake duration})})$

This calculation cannot be performed precisely due to the lack of raw data, but it is possible to use the estimates for the k_2 value obtained above to estimate a value for the assimilation efficiency. The value of the concentration in fish at time zero of the depuration phase (C_0) can be estimated to be around 0.0059 µg/g (lipid?) for anti-DP, and around 0.0075 µg/g (lipid?) for syn-DP, from the plot of ln [back-calculated concentration] versus time. As mentioned above it is not clear if these are lipid-normalised concentrations or not. Using the known concentrations in food (1.17 µg/g (lipid weight) for the anti- isomer and 0.79 µg/g (lipid weight) of the syn- isomer), the values of the overall depuration rate constants estimated above (0.027 d^{-1} for anti-DP and 0.020 d^{-1} for syn-DP) and the feeding rate[23], the assimilation efficiency can be roughly estimated as 0.008 (0.8 %) for anti-DP and 0.016 (1.6 %) for syn-DP. These values are significantly lower than estimated by Tomy *et al*. (2008).

- Depuration half-life was calculated by the formula ln(2)/depuration rate constant. This is the same as OECD 305, except that for the latter, the growth-corrected depuration rate constant can be used. As the authors seem to have corrected the data for growth prior to analysis, then presumably the derived half-life is effectively already growth-corrected (see above and as also assumed by Arnot & Quinn, 2015).

- The equilibrium biomagnification factor (BMF) was predicted from the equation (assimilation efficiency × feeding rate) / depuration rate constant, where the feeding rate is "corrected for the lipid percentage of the food". The OECD TG 305 uses the same equation, with lipid correction for the feeding rate where lipid-corrected concentration data have been used. As the authors seem to have corrected the data for lipid prior to analysis, then presumably the reported BMFs can be considered to be lipid-normalised. However, as noted above the method used by Tomy *et al*. (2008) to estimate the assimilation efficiency is different to that recommended in OECD TG 305. This may be important when comparing the data with the results of other substances.

- For the uptake phase, Tomy *et al.* (2008) appear to report rates (in units of nmoles/day) rather than the rate constant (which has units of g/g/day). It is, however, possible to estimate an approximate rate constant for the uptake (k_1) from the initial rate of uptake[24]. Using the reported initial rates of uptake of 0.018 nmol/day for anti-DP and 0.045 nmol/day for syn-DP, the equivalent values of k_1 can be estimated as 0.0002 g/g/day for anti-DP and 0.00075 g/g/day for syn-DP. These values of k_1 correspond to an assimilation efficiency of approximately 0.02 (2 %) for anti-DP and 0.075 (7.5 % for syn-DP). Assuming the growth-corrected depuration rate constant (k_{2g}) is around 0.019 d^{-1} for anti-DP and 0.012 d^{-1} for syn-DP, the kinetic, growth-corrected BMF_{kg} can be estimated to be around 0.01 for anti-DP and 0.062 for syn-DP. Normalising these values to the lipid contents in fish at day 49 and the lipid concentration in food, gives the growth-corrected and lipid normalised BMF_{kgL} as around 0.022 for anti-DP and 0.12 for syn-DP. However, it should be noted that it is not clear how the raw data were lipid-normalised in the original study and so these lipid-normalised values should be treated with caution.

[23] The feeding rate was 0.01 kg food/kg fish (1 % of body weight); if the concentrations are on a lipid basis then the feeding rate on a lipid basis is needed for the calculation. Using the food lipid content of 14.3 % and the lipid content of fish on day 49 of uptake of 6.9 % for anti-DP and 7.5 % for syn-DP the equivalent feeding rate on a kg lipid food/kg lipid fish can be estimated to be around 0.021 for anti-DP and 0.019 for syn-DP.

[24] The rate of uptake = $k_1 \times [C_{food}] - k_2 \times [C_{fish}]$. At the early stages of the uptake where the concentrations in fish are low the term $k_2 \times [C_{fish}]$ is small compared with the term $k_1 \times [C_{food}]$ and so the intial rate of uptake approximates to $k_1 \times [C_{food}]$.

Whilst the study is considered to be reliable with restrictions by the Registrant, access to the original raw data is required for proper interpretation. An attempt to contact the lead author was unsuccessful, and so a number of assumptions and approximations have had to be made in order to attempt to calculate the appropriate kinetic parameters from the data. This has necessarily introduced some uncertainty into the analysis but the results of re-analysis suggests that the actual BMF from the study is much lower than originally reported by Tomy *et al.* (2008). The main findings of the re-analysis are summarised below:

	Anti-DP	**Syn-DP**
Growth-corrected depuration rate constant	~0.019 d^{-1}	~0.012 d^{-1}
Growth-corrected depuration half-life	~36 days	~58 days
Assimilation efficiency	~0.8-2 %	~1.6-7.5 %
Growth-corrected BMF	~0.01	~0.062
Growth-corrected and lipid-normalised BMF	~0.022	~0.12

This study has also been analysed in depth by Arnot & Quinn (2015) who critically reviewed a large number of published dietary feeding studies (based on information in the original publications rather than primary data). Arnot & Quinn (2015) state that the BMFs derived by Tomy *et al*. (2008) are not reproducible: by reading the depuration data from Figures 1 and 2 of the original paper (which plot the (control- and lipid-corrected) concentration of each isomer in nanomoles for whole body (minus liver) tissues against time in days), and transforming them (using natural logarithms) to re-calculate the depuration rate constant, growth-corrected kinetic BMFs of 0.046 and 0.023 were estimated for the syn- and anti- isomers, respectively. These are reasonably consistent with the values estimated above. The re-estimated growth-corrected depuration rate constant and half-life for the syn- isomer were consistent with those provided in Tomy *et al*. (2008), but the re-estimated growth-corrected depuration rate constant for the anti-isomer was slightly lower at 0.017 d^{-1}, with a higher growth corrected depuration half-life of about 40 days (again these values are reasonably consistent with the values estimated above). Arnot & Quinn (2015) also estimated lipid-normalised growth-corrected BMFs, but given the uncertainty in how the lipid normalisation was done in the original paper, these values are not indicated here (they are slightly higher than the BMFs without lipid normalisation).

Tomy *et al*. (2008) noted that in view of the high log K_{OW} of Dechlorane Plus and a BMF greater than 1, it is anticipated that Dechlorane Plus isomers will biomagnify in aquatic food webs. In contrast, the analysis presented in this report indicates that the BMFs actually derived by Tomy *et al.* (2008) are unreliable and so they are not considered further in this dossier.

Conclusions

Overall the Dossier Submitter agrees with the Registrant's assignment of the Tomy *et al*. (2008) study as "reliable with restrictions". Unfortunately it is not possible to fully analyse the study in line with the recommendations of OECD TG 305-III since the raw data are not available. Nevertheless, the calculations made in this dossier and by Arnot & Quinn (2015) indicate estimated growth-corrected BMFs of 0.01 – 0.023 for the anti- isomer and 0.046 – 0.062 for the syn- isomer. Lipid normalisation of these data is uncertain but it is possible that the lipid-normalised values would be around a factor of two higher than these values. Another factor that should be considered is that the study used a feeding rate of 1 % of body weight per day. OECD TG 305 recommends a feeding rate of 1-2 % of body weight. In theory, the BMF obtained in an

OECD TG 305 study should be directly proportional to the feeding rate[25], but even if a higher feeding rate of 2 % of body weight had been used, the BMF would still likely be below 1 (i.e. around 0.12 for syn-DP, which has the higher BMF).

The following points are important:

- Neither isomer had reached a steady-state concentration in the fish after 49 days of uptake, implying that concentrations could become higher given sufficient exposure time. However, the kinetic approach used above (and presumably by Arnot & Quinn, 2015) would approximate to the steady-state situation. Using the overall depuration rate constants (k_2) of 0.027 d^{-1} for anti-DP and 0.02 d^{-1} for syn-DP, it can be estimated that it would take approximately 110-150 days to achieve 95 % steady state.
- The growth-corrected depuration half-lives are around 30 – 40 days for the anti- isomer and 50 – 70 days for the syn- isomer for juvenile Rainbow Trout (*O. mykiss*).
- The maximum arithmetic mean fish whole body (minus liver) (control- and lipid- corrected) mass of the syn- isomer present at the end of the uptake period was 2.2 nmole, or 1.44 µg (value read from a graph). The mean mass of the anti- isomer was 1.7 nmole, or 1.11 µg per fish (value read from a graph). Fish weight was around 51.5 g, but a wet weight concentration cannot be estimated as the lipid correction method is unclear.

Turning to the other studies summarised in Appendix 1, the non-guideline study by Zitko (1980) with Atlantic Salmon *Salmo salar* is not valid for a variety of reasons. It does, however, indicate that uptake and elimination can occur in fish. The highest reported fish concentration was 176 µg/kg ww after 15 days, although it is not clear whether this included substance present in the gut or adsorbed to skin (whole fish were analysed). A depuration half-life of 58 days was estimated by the study authors, but this did not take any account of fish growth or lipid content changes. The fish growth rate for this part of the study was 0.19 d^{-1} (based on a plot of fish weight against time from day 16 of depuration). If the results are expressed in terms of mass of Dechlorane Plus, there was effectively *no change in the amount of Dechlorane Plus present in the fish during the 72-day depuration phase* (the range was 290-370 ng, with the lower value occurring after 16 days of depuration). Arnot & Quinn (2015; supporting information) estimated a growth-corrected depuration half-life of about 100 days for ca. 15 g, 5 % lipid content fish using the results of this study. It therefore supports the findings of a long elimination half-life by Tomy *et al*. (2008).

Xiao *et al*. (2013) found that juvenile Rainbow Trout *O. mykiss* appear to absorb at least 16 % of the dose from a single feed (a single exposure to 1 270 µg/kg in food led to a whole body concentration of around 12 µg/kg), although there are analytical uncertainties with this study. The study of Zeng *et al*. (2014a) showed that a steady state was not achieved over 50 days' exposure in Common Carp *Cyprinus carpio* muscle, serum or liver tissue, with the highest concentrations being detected at the end of the 50-day uptake period (when the total concentration in liver was ~615 µg/kg ww). This further supports the findings of the Tomy *et al*. (2008) study. The substance also accumulated in gonad tissue. This study suggests that factors other than lipid solubility (e.g. hepatic binding enzymes) could play important roles in determining deposition. Whole body concentrations were not reported and the small number of samples and variable depuration results also prevent any calculations of an overall depuration half-life. A BMF therefore cannot be derived from the data. However, residues remained in all tissues after 45 days of depuration (e.g. the total concentration in liver was ~250 µg/kg ww at the end of the study) which is consistent with the relatively long depuration half-life seen in other studies.

Benchmarking the fish bioaccumulation data

- Inoue *et al.* (2012) investigated the correlation of dietary BMF with BCF in Common

[25] The BMF = feeding rate × assimilation efficiency/depuration rate constant. Thus assuming the assimilation efficiency and depuration rate constant do not vary much with feeding rate, doubling the feeding rate should double the BMF.

Carp *Cyprinus carpio* for eight aromatic compounds with log K_{OW} values in the range 4.3 – 9.0. This indicated that a BMF (growth-corrected and lipid-normalised) above 0.31 can correspond to a (lipid normalised) BCF above 5 000 L/kg. A theoretical analysis by MacKay *et al*. (2013) provides information to suggest that BCF values of 5 000 and 2 000 L/kg correspond approximately to BMF values of 0.25 and 0.1, respectively. This assumes that chemicals partition to the lipid fraction of the fish with no metabolism or growth dilution, and "sets" values for respiration rate and absorption efficiency that may not be typical for all species. Although a simplification, this supports the empirical relationship reported by Inoue *et al*. (2012). The re-estimated BMFs from the Tomy *et al*. (2008) study do not correlate with a BCF above 5 000 L/kg. A theoretical BMF of around 0.092 – 0.12 for the syn- isomer (if the feeding rate had been 2 % of body weight rather than 1 %) would just about correlate with a BCF of 2 000 L/kg. Whilst it is possible that lipid normalisation could increase the BMF still further (perhaps up to about 0.24), this cannot be established with any certainty.

- In the absence of a reliable aqueous fish BCF value to compare to the REACH Annex XIII criteria, the depuration half-life is considered further. There may be significant variability between and within species (e.g. due to differences in lipid content and metabolic profiles). However, advantages of using this metric as a key indicator for bioaccumulation assessment are that it is relatively easy to determine and not so dependent on other variables within a particular study. The achievement of a high level of bioaccumulation is obviously related to uptake as well as depuration kinetics, since this affects body burdens. Nevertheless, in terms of the aims of protecting organisms from unpredictable adverse effects, a long depuration half-life is a key determinant of bioaccumulation potential and is directly related to one of the protection aims of the PBT assessment, i.e. concentrations for a PBT substance may take a long time to decline once emissions cease.

Two benchmarking approaches have been used to compare the depuration half-life of Dechlorane Plus with other relevant substances:

(a) An Environment Agency report (EA, 2012) analysed depuration rate constants (k_2) from a large number of available BCF studies. A k_2 value ≤ 0.065 d^{-1} (95 % CI: 0.062 – 0.068) or a lipid-normalised k_2 ≤0.085 d^{-1} 95 % CI: 0.083 – 0.086)[26] was found to be consistent with a BCF of ≥5 000 L/kg (normalised to a 5 % lipid content)[27]. The 'growth-corrected lipid-normalised' k_2 values for Dechlorane Plus from Tomy *et al*. (2008) were 0.010 – 0.013 d^{-1} (syn- isomer) and 0.017 – 0.023 d^{-1} (anti- isomer). Thus the low rate of depuration seen in the feeding study with *O. mykiss* is consistent with a BCF above 5 000 L/kg.

(b) It is relevant to compare the data for Dechlorane Plus with polychlorobiphenyls (PCBs) and other substances previously assessed to be vB under REACH. In particular, PCB data were used to support the identification of perfluorohexane-1-sulfonic acid and its salts (PFHxS, CAS no. 355-46-4) as vB (the data are summarised in Appendix 7). There are nine studies, although only four provide useable depuration half-lives for this analysis. Twelve further substances had been determined as meeting the vB criteria up to May 2015. Seven studies covering four of those substances report a depuration half-life (see Appendix 6). The information is summarised in Table 9.

Table 9: Depuration half-lives and BCF values for chemicals meeting the vB criteria

[26] Equivalent to depuration half-lives above around 8-10 days, assuming first order kinetics.

[27] Goss *et al*. (2013) also consider the use of elimination half-life data in bioaccumulation assessment, taking a first principles approach without considering actual data. The proposed half-life corresponding to a BMF of 1 is 70 days assuming 100 % assimilation efficiency (longer if the assimilation rate goes down).

Depuration half-life, days	BCF value, L/kg	Source
39 – 77	7 273	SCCPs (REACH vB)
77 – 87		
2.8 – 4.2	3 730 – 10 500	Musk xylene (REACH vB)
3.8, 105	≥11 495	D4 (REACH vB)
24 – 39	≥ 5 860	D5 (REACH vB)
19 – 22	12 600	
>28	>>5 000	Arochlor 1248 (commercial PCB mixture)
>42, 50		Arochlor 1260 (commercial PCB mixture)
>974		PCB-52

Therefore with one exception (musk xylene), substances already agreed to meet the vB criterion or identified as POPs based on BCF values generally have relatively long depuration half-lives of 20 days or more in at least one fish species. A depuration half-life above around 8-10 days is also suggestive of a lipid-normalised and growth-corrected BCF above 5 000 L/kg according to the analysis in EA (2012). The depuration half-life for Dechlorane Plus was around 30-40 days for the anti- isomer and 50-70 days for the syn- isomer in the dietary study of Tomy *et al*. (2008) with juvenile Rainbow Trout *O. mykiss*.[28] Zeng *et al*. (2014a) found that residues remained in all tissues in a study using Common Carp *C. carpio* after 45 days of depuration. The long depuration half-life for Dechlorane Plus in fish is therefore highly indicative of a very bioaccumulative substance.

As noted above depuration half-life can be influenced by a number of experimental factors such as species differences, age of fish, etc. Therefore it may be difficult to reach clear conclusions for borderline cases. Sufficient uptake of a chemical by an organism is also required, so depuration half-life alone may not be indicative of a very bioaccumulative substance. However, field monitoring data (see following sections) suggest that Dechlorane Plus is bioavailable, and can achieve a relatively high body burden in some cases that may be expected to be associated with toxic effects due to baseline narcosis (see Appendix 3, 4 and 6). Therefore the use of depuration half-life to guide decision-making is considered to be reasonable for Dechlorane Plus, and its very long depuration half-life is given a high weighting in the bioaccumulation assessment.

3.4.1.2.3 Sediment exposure

Li *et al.* (2014a) determined the bioaccumulation of Dechlorane Plus in the oligochaete *Lumbriculus variegatus*. The study was carried out using both laboratory-spiked sediment and field-collected sediment from an electronics recycling site in South China. The test was carried out in triplicate beakers containing approximately 120 g of wet sediment and 300 mL of moderately hard water, with each beaker containing 30 worms. The mean measured concentrations of anti-DP and syn-DP in the field sediments were 8.8 and 3.54 mg/kg organic carbon, respectively. For the laboratory-spiked sediments two exposure concentrations (high and low) were used for each of anti-DP and syn-DP. The mean measured anti-DP concentrations were 83.1 mg/kg organic carbon (high) and 24.3 mg/kg organic carbon (low). The mean measured syn-DP concentrations were 27.3 mg/kg organic carbon (high) and 7.66 mg/kg organic carbon (low). An uncontaminated sediment was used as a control. The organic carbon contents of the sediments were 2.75 % for the control and 1.80 % for the field sediment. The organic carbon

[28] Arnot & Quinn (2015) estimated a growth-corrected depuration half-life of about 100 days for a ca. 15 g fish with 5 % lipid content using the results of the Zitko (1980) study with Atlantic Salmon *S. salar*. However, this study is not valid for a variety of reasons: in particular co-exposure to other related substances might have affected the uptake or metabolism of Dechlorane Plus, as well as the health of the fish.

content of the laboratory soil is not clear.

The test consisted of a 28-day uptake period followed by a 28-day (laboratory sediment) or 21-day (field sediment) depuration period. The test was carried out at 23 °C and the worms were not fed during the test. Approximately 150 mL of the overlying water was changed twice daily. The dissolved oxygen content of the water was 5.4±0.4 mg/L and the pH was 7.6. The sediment concentrations were found to remain constant during the uptake phase of the test.

The exposed worms behaved similarly to the control worms, with no overt avoidance of the sediment and no significant reproduction of the worms occurred.

The concentrations of anti-DP and syn-DP in the worms increased during the first 14 days and then reached a plateau (steady-state) between days 14 and 28. The biota-sediment accumulation factor (BSAF) was determined as the ratio of the uptake rate constant and the depuration rate constant obtained using a first order kinetic model. No obvious growth of the worms was reported to occur in the test (worm weights per replicate were similar before and after the test).

The uptake rate constants for anti-DP were determined to be between 0.027 and 0.037 g organic carbon/g lipid/day in the laboratory sediments and 0.037 g organic carbon/g lipid/day in the field sediment. The depuration rate constants for anti-DP were determined to be 0.092-0.095 d^{-1} in laboratory sediment and 0.18 d^{-1} in the field sediment and the BSAF was determined to be 0.29-0.39 g organic carbon/g lipid in laboratory sediment and 0.21 g organic carbon/g lipid in the field sediment. For syn-DP the uptake rate constant was between 0.0298 and 0.0698 g organic carbon/g lipid/day in the laboratory sediments and 0.044 g organic carbon/g lipid/day in the field sediment, and the depuration rate constant was 0.063-0.15 d^{-1} in the laboratory sediments and 0.13 d^{-1} in the field sediment. The BSAF values for syn-DP were therefore 0.47-0.48 g organic carbon/g lipid in the laboratory sediments and 0.34 g organic carbon/g lipid in the field sediment.

All of the BSAF values determined were <1. The BSAF value for syn-DP was greater than for anti-DP and this difference was statistically significant ($p<0.05$) for the low-level laboratory sediment and the field sediment.

Discussion

The BSAF determined with *Lumbriculus variegatus* is in the range 0.21-0.39 g organic carbon/g lipid for anti-DP and 0.34-0.48 g organic carbon/g lipid for syn-DP. The BSAF for syn-DP was found to be higher than for anti-DP. The values obtained in the laboratory study are discussed further in Section 3.4.1.2.4 alongside the available BSAF values from field studies.

3.4.1.2.4 Field studies

Twenty-two biota monitoring studies that report some measure of bioaccumulation potential have been summarised in detail in Appendix 1 (Wu *et al*., 2010; Tomy *et al*., 2007; Mo *et al*., 2013; Klosterhaus *et al*., 2012; Peng *et al.*, 2012; He *et al*., 2014; Zhang *et al*., 2011b; Peng *et al*., 2014; Wang *et al*., 2015; Barón *et al*., 2013; She *et al*., 2013; Jia *et al*., 2011; Shen *et al*., 2011a; Wang *et al.*, 2012; Chen *et al*., 2012b; Muñoz-Arnanz *et al*., 2012; Zhang *et al.*, 2010b; Kim *et al.*, 2015; Wu *et al.*, 2013; Li *et al.,* 2014b; Sühring *et al*., 2015; Sun *et al.*, 2015). Other biota monitoring data are reported in Appendix 3.

In general, monitoring studies are of limited usefulness for bioaccumulation assessment because the concentrations that the organisms were exposed to are unknown. Some species are highly migratory, so exposure at the sampling location might not be very relevant (i.e. the body burden may result from exposures some distance away). The studies are often screening exercises, with several substances investigated in the same samples, and often only include relatively low numbers of samples. The lack of a suitably ring-tested analytical method means that the results should be treated with caution, especially when comparing studies undertaken by different laboratories and at different times. Comparisons are also limited by the expression of the

concentration in terms of either wet or lipid weight (rarely both), the use of different tissues and potentially the age/size and health of the organism when it was collected (particularly dead or injured individuals that may have depleted fat reserves). This is important if the accumulation of Dechlorane Plus is linked to more factors than lipid content alone, as suggested by the studies of Zeng *et al*. (2014a), de la Torre *et al*. (2012), Zhang *et al*. (2011a), Li *et al*. (2013a), Chen *et al*. (2013b) and Zheng *et al*. (2014a) (summarised in Section 3.4.1).

Nevertheless, the available monitoring studies show that the two isomers of Dechlorane Plus are widely dispersed in the European environment. Evidence from around the world shows that uptake can clearly occur in a wide range of wildlife species throughout freshwater and marine aquatic food webs, from invertebrates and fish to piscivorous top predators such as Bald Eagle *Haliaeetus leucocephalus*, Double-crested Cormorant *Phalacrocorax auritus*, Eurasian Otter *Lutra lutra* and cetaceans (e.g. *Delphinus delphis*). Dechlorane Plus is also found in bird- and mammal-eating predators associated with aquatic environments (such as Peregrine Falcon *Falco peregrinus* and Polar Bear *Ursus maritimus*). From the small number of studies available, maximum levels in top predators in Europe are broadly in the range 0.1 – 1 µg/kg ww, although there are many non-detects.

Most European samples have been collected from relatively unpolluted environments, where the substance is more likely to be present at very low concentrations due to long range transport rather than (in)direct local releases. Such studies show that Dechlorane Plus can be present in wildlife (including top predators) in regions relatively remote from human activity, although the levels are typically very low (<0.1 µg/kg ww). In contrast, studies in more polluted environments in China and near the U.S. manufacturing facility show higher levels, indicating that organisms have the potential to accumulate in the region of 1 – 10 µg/kg ww, and up to 1 mg/kg ww [97 mg/kg lw] in fish muscle in the vicinity of point sources (Wang *et al*., 2015) (whole body concentrations are not available). This suggests that if exposure in the European environment were to increase, levels in wildlife might also increase. The higher levels that have been found in wildlife exceed concentrations that are considered to be of concern within a bioaccumulation context (e.g. a "critical concentration" of 0.065 or 0.65 mg/kg ww [1.3 or 13 mg/kg lw] can be estimated for Dechlorane Plus based on a critical body burden of 0.001 mmol/kg ww recommended in the REACH PBT Guidance (ECHA, 2017a); see Appendix 7).

According to equilibrium partitioning theory, BSAF values should be in the range 1–2 when the partitioning of a non-ionic organic chemical is at equilibrium between the organic carbon in the sediment and lipids of an organism (e.g. Burkhard *et al*., 2004). Lower BSAFs imply that the partitioning of an organic compound into lipids is lower than expected, i.e. that the partitioning between the fish or invertebrate species and the sediment is not in equilibrium (which may be due to chemical disequilibrium between the water and sediment, limited bioavailability due to the amount and type of sediment organic carbon and/or dietary uptake efficiencies, and/or metabolism in the organism or its food items). In contrast, higher BSAFs suggest that the substance can accumulate to a greater extent than by simple partitioning alone. The available data for Dechlorane Plus are as follows:

- For fish, reported average BSAFs[29] for total isomers were below 1 in four studies (Zhang *et al*. (2011c): three species; Shen *et al*. (2011a): one fish species; Wang *et al.* (2012): one fish species; He *et al*. (2014): three species). However, another study (Wang *et al*., 2015) reported BSAFs above 1 for three fish species, with values up to 3.2 and 9.0 for the anti- and syn- isomers, respectively.
- For invertebrates, one study reported BSAFs below 1.7 (Wang *et al.* (2015): river snail & shrimp) whereas another reported BSAFs above 2 (Jia *et al*. (2011): oysters).
- BSAF values below 1 (in the range 0.2-0.5 g organic carbon/g lipid) have been

[29] Fish BSAFs are defined as the lipid normalized concentration in the fish divided by the organic carbon normalized sediment concentration.

determined for *Lumbriculus variegatus* in a laboratory study (Li *et al*. (2014a); see Section 3.4.1.2.3).

This conflicting information is difficult to interpret. The studies tend to be based on small sample sizes, with sediment and biota sometimes collected at different time points and/or locations in the same general area, so their reliability is uncertain. In addition, the ECHA PBT guidance R.11 (Appendix R.11-4) states that it is often difficult to distinguish between real uptake and adsorption to the organisms or interference of gut content in the determination of the BSAF values for highly hydrophobic substances. BSAF values of 0.1 and below may give an incomplete indication of bioaccumulation potential based on pore water concentrations. However, one study for fish and one for invertebrates suggest that BSAFs might exceed 2, suggesting an enhanced level of bioaccumulation. For two studies where the BSAF is below 1, the BSAF values for BDE-183 (a heptabromodiphenyl ether) are similar to those for Dechlorane Plus in the same samples. In the support document for bis(pentabromophenyl)ether, heptaBDE congeners were concluded to meet the B criterion of Annex XIII (ECHA, 2012a).

Detection of Dechlorane Plus (and anti-[DP-1Cl]) in eggs shows that maternal uptake and transfer to eggs can occur in birds. Similar maternal uptake and transfer has also been seen in fish (e.g. Wu *et al.* (2013) and Sühring *et al.* (2015)). The studies of Zhang *et al*. (2011a) and Zeng *et al*. (2014a) also show that the substance can cross the blood-brain barrier and be passed from females to eggs in fish. Li *et al.* (2014b) showed that the substance can cross the blood-brain barrier in frogs. Sensitive life stages and tissues are therefore exposed to the substance.

Of particular relevance to this assessment are field studies that indicate biomagnification between trophic levels. The interpretation of such studies is evolving and it is clear that they can be complicated by a range of factors such as migratory behaviour of the species sampled, difficulties in establishing trophic position and feeding relationships, concentration gradients in water and/or sediment, and measurement limitations (e.g. in terms of temporal and spatial coverage, sample numbers (especially for larger species), specific tissue versus whole body, contamination during sampling, processing and analysis, etc.). These findings should therefore be treated with caution. Whilst some studies do not provide convincing evidence of biomagnification (e.g. Barón *et al*. (2013), Peng *et al*. (2014) and Klosterhaus *et al*. (2012)), four independent studies of aquatic food webs provide some evidence of trophic magnification:

- Wu *et al*. (2010) calculated a TMF above 1 for both Dechlorane Plus isomers in a Chinese aquatic food web. The value for both isomers together was approximately five times higher than that of total PBDEs and similar to polychlorinated biphenyls in the same web (although the overall Dechlorane Plus concentrations were 1 – 2 orders of magnitude lower). Sample numbers were low. The TMF depends on the assignment of one species (a water snake) to the highest trophic position; inclusion of a top predatory fish (Northern Snakehead) results in a lack of biomagnification (and even biodilution for the anti- isomer). Levels in three benthic-feeding carp species are consistently higher than potential invertebrate prey, suggesting that biomagnification may occur for some feeding relationships although this might also be influenced by sediment ingestion. This study provides evidence of biomagnification for some feeding relationships in an aquatic food web, though this is not unequivocal.
- Tomy *et al*. (2007) investigated food web samples from Lake Ontario and Lake Winnipeg, Canada. In Lake Winnipeg, trophic level-adjusted BMFs (based on the ratio of lipid-corrected concentrations) for four feeding relationships were all below 1 for both syn- and anti- isomers, with the exception of the Walleye/Whitefish predator-prey relationship for the anti- isomer only (BMF of 11). In Lake Ontario, trophic level-adjusted BMFs for three of four feeding relationships were below 1 for both isomers. The exception was the Lake Trout/Smelt predator-prey relationship, with a BMF of 12 for the syn- isomer and 11 for the anti- isomer. These calculations assume that a predator consumes one prey species only, and as this is unlikely to be the case in reality, the BMFs should be treated with caution.

  A TMF value above 1 was calculated for the anti- isomer in Lake Winnipeg (TMF = 2.5, r^2

= 0.12, p = 0.04), but not for the syn- isomer, or either isomer in Lake Ontario. The positioning of some of the species in the food web is open to question, and the Lake Winnipeg results were based on fish muscle only, which introduces uncertainty. Low concentrations, the very small sample sizes and possibility of variable exposure raise additional concerns about the representivity of the data for these food chains.

Whilst the study suggests that interspecies differences in bioaccumulation/ biotransformation are likely, and that biomagnification may be taking place in some aquatic food webs and feeding relationships, the evidence is equivocal.

- Wang *et al*. (2015) investigated trophic magnification in an aquatic food chain that receives discharges from the Dechlorane Plus production facility in China. Sample numbers were low, but the TMF was 1.9 (95 % CI: 1.1–3.4) for anti-Dechlorane Plus, and this was statistically significant. Total Dechlorane Plus concentrations were in the range 0.8 – 1.1 mg/kg ww for four of the five fish species sampled, including the species at the highest trophic level.

  In addition, the log-normalized concentration ratios of anti-DP-1Cl to anti-DP showed a significant relationship with trophic level (p < 0.001), implying that anti-DP-1Cl may have a higher trophic magnification potential than anti-DP.

  A significant negative correlation was found between f_{anti} and trophic level for the sampled species (p < 0.01), suggesting that the organisms have lower uptake efficiencies and higher depuration rates for the anti- isomer compared to the syn-isomer.

- Sun *et al.* (2015) investigated the bioaccumulation of Dechlorane Plus in biota from the Island Mangrove Nature Reserve, Pearl River estuary, South China. A total of 22 samples from four species were analysed in the study and anti-DP was detectable in 100 % of the samples and syn-DP was detectable in 54 % of the samples. The TMF for total Dechlorane Plus was determined to be 2.31, although the regression was not statistically significant (p = 0.07). Estimated BMFs for various predator-prey combinations were in the range 1.27 to 11.8. These calculations assume that a predator consumes one prey species only, and as this is unlikely to be the case in reality, the BMFs should be treated with caution.

The interpretation of trophic transfer studies involving waterbirds is complicated by the fact that sample sizes are small, and a single tissue (usually egg, muscle or liver) is typically analysed. Zheng *et al*. (2014a) (summarised in Section 3.4.2) showed that fat tissue is an important compartment for Dechlorane Plus accumulation in the Domestic Chicken (*Gallus gallus*), so whole body burdens might be significantly different from those implied by measured concentrations in a single tissue (particularly muscle). Coupled with the uncertainties about the relevance of lipid normalisation, firm conclusions about trophic transfer in birds cannot be drawn from the available data.

3.4.2 Bioaccumulation in terrestrial organisms (soil dwelling organisms, vertebrates)

3.4.2.1 Screening information

Although there are significant uncertainties in the physicochemical data set, the combination of a predicted log K_{OW} >2 and log K_{OA} >5 indicate that Dechlorane Plus has the potential to bioaccumulate in terrestrial food webs if the rate of chemical transformation or metabolism is low, as suggested by Gobas *et al*. (2003) and Kelly *et al*. (2007). In particular, bioaccumulation of very hydrophobic substances (log K_{OW} >7) in terrestrial biota does not necessarily fall off with increasing K_{OW}. It is therefore important not to overlook the potential for terrestrial bioaccumulation for Dechlorane Plus.

It is not useful to estimate an earthworm BCF using QSAR approaches given the uncertainty in the log K_{OW} value and limitations of the QSAR equations themselves.

3.4.2.2 Laboratory studies

Short-term and *in vitro* studies are summarised in Appendix 1 (Zhang *et al*., 2014; Chabot-Giguère *et al*., 2013; Peng *et al*., 2014; Zhang *et al*., 2015). Due to limitations in terms of duration or experimental set up, these do not provide any relevant information for the assessment of bioaccumulation.

Mammalian studies are reported in Section 4.1.

Three studies involving birds are available and are summarised below:

- Li *et al*. (2013a) exposed male Common Quail (*Coturnix coturnix*) (6–8 weeks' old, with an average weight of 125 g) to commercial Dechlorane Plus mixed in corn oil by oral gavage for 90 days at different doses (0, 1, 10, and 100 mg/kg/d). Sixty animals were used in all. Liver, muscle and serum samples were analysed using GC-MS at the end of the exposure period. The LoDs for the anti- and syn- isomers were 46.12 and 27.85 µg/kg lw in muscle/liver, and 0.054 and 0.20 ng/mL in serum, respectively.

  Both isomers were detected in all of the tissues measured in the control group at the end of the 90-day exposure period (average concentrations in liver were 15 ± 8.1 and 10 ± 5.9 mg/kg lw for the anti- and syn- isomer, respectively), indicating a background level of exposure (e.g. from feed or air-borne dust). DP-1Cl was also detected in the control group (e.g. 0.131 mg/kg lw in liver).

  In the exposure groups, the highest concentrations were detected in liver. For the 10 mg/kg/d dose group, the total liver concentration was ~1 750 mg/kg lw (260 ± 39 mg/kg lw for the anti- isomer and 1 500 ± 69 mg/kg lw for the syn- isomer). These levels were around ten times greater than in the lower dose group, and three times greater than in the highest dose group (although the levels of the anti- isomer were greatest in liver in the 1 mg/kg/d group, at 300 ± 120 mg/kg lw). The syn- isomer tended to accumulate more in the two highest dose groups, whereas the anti- isomer was dominant in the low-dose group (the isomer ratio was similar to the commercial product at this dose).

  The highest average total concentration of the mono-dechlorination products syn-DP-1Cl and anti-DP-1Cl occurred in the 10 mg/kg/d group, reaching 1.2 mg/kg lw in liver. Two additional unidentified substances were detected in the tissue samples as well as in the commercial substance.

  In conclusion, both isomers preferentially accumulate in liver rather than muscle or blood in Common Quail, but accumulation was not dose-dependent (with lower levels observed in the top dose group compared to the mid-dose group). There appeared to be enrichment of the syn- isomer at higher exposures. It is not known how long it would take to achieve a steady state.

- Zheng *et al*. (2014a) investigated dietary accumulation and tissue distribution in Domestic Chickens (*Gallus gallus domesticus*). Birds purchased from a market (several weeks old; n=12: 1 male, 11 females) were raised for seven months in a farmer's yard surrounded by e-waste recycling workshops in Qingyuan county, Guangdong province, China. Atmospheric particles (n=10, collected using a high-volume air sampler), chicken food (mixture of rice, wheat and other types of grain, n=9) and soil (n=10) samples were collected from the yard. After sacrifice, eight tissues (liver, muscle, heart, gonad, brain, lung, and fat from all birds, and kidney from ten) and digestive tract contents (n=12 for chyme[30], intestinal contents and faeces) were sampled, weighed and then stored at –20 °C until further analysis.

  After being spiked with surrogate standards (BDE-77, -181 & -205), approximately 2 g of lyophilized tissue sample were extracted with solvents, then spiked with known amounts of internal standards (BDE-118 & -128, 4-F-BDE-67 and 3-F-BDE-153) prior to instrumental analysis via GC-MS using electron capture negative ionization operated in the selected ion

[30] Chyme is partly digested food that is expelled by the stomach into the duodenum.

monitoring mode.

Regular analysis of procedural blanks (n=17), spiking blanks, blind triplicate samples, and regular injection of solvent blanks and standard solutions were performed for quality control purposes. Trace amounts of the anti- isomer were detected in procedural blanks with median concentrations of 0.46 ng/mL (accounting for 15 % of the sample with the lowest Dechlorane Plus level), so this was subtracted from the sample results. The recoveries in the spiking blanks were 90 ± 6.5 %. No surrogate correction was made to the final concentrations. Target chemicals detected in the triplicate samples were consistent (RSD <15 %). LoQs for syn-DP, anti-DP, anti-DP-2Cl, and anti-DP-1Cl were 0.04, 0.12, < 0.01, < 0.01 µg/kg dw/ww in environment matrices; 0.21, 0.61, 0.01, 0.01 µg/kg dw/ww in digestive tract; and 0.11, 0.13, 0.01 and 0.01 µg/kg dw/ww in chicken tissues, respectively.

The median (and range) concentrations of Dechlorane Plus (total isomers) in chicken food, atmospheric particulates and soil were 0.2 (not detected – 0.98), 1 500 (360–8 700), and 7 600 (5 900–10 000) µg/kg dw, respectively.

The median (and range) concentrations of Dechlorane Plus (total isomers) in chyme, intestinal contents and faeces were 7.6 (3.7–1 100), 35 (14–580), and 31 (not detected–1 300) µg/kg dw, respectively. Mass balance calculations indicated that there was 52 % "absorption" [transfer?] from chyme to intestinal contents and 50 % "absorption" [transfer?] from intestinal contents to faeces. No significant differences in Dechlorane Plus levels were found between the intestinal contents and faeces (p > 0.05); while levels in chyme were significantly lower (p < 0.05).

The substance was detected in all tissues. Since there was only one male, gender differences in accumulation behaviour were not considered. Median Dechlorane Plus (total isomers) concentrations (and range) were in the order of fat (10 (3.4–72) µg/kg ww) > gonad (7.9 (0.82–417) µg/kg ww) > kidney (6.8 (1.07–460) µg/kg ww) > heart (6.3 (1.0–21) µg/kg ww) > liver (4.4 (0.79–320) µg/kg ww) > lung (2.8 (1.3–126) µg/kg ww) > brain (1.4 (0.43–5.2) µg/kg ww) > muscle (0.92 (not detected–124) µg/kg ww). The range for all tissues was 17 – 140 µg/kg lw.

The mass deposition percentage of Dechlorane Plus in individual tissues indicated that fat was the most important reservoir (accounting for 56 % of the residues), followed by liver (12 %) and gonad (12 %). No significant differences were found amongst the tissues levels (p > 0.05), except muscle contained significantly lower concentrations than those in liver, gonad, fat, and kidney (p < 0.05). This was thought to relate to the lower lipid content of muscle.

The lipid content of fat tissue was significantly higher than other tissues (p < 0.05) although fat did not show significantly elevated substance concentrations compared with other tissues (expect for muscle). This suggests that lipid content was not the only factor that influenced tissue distribution, and it was speculated that blood perfusion and sequestration by proteins could be additional factors (Government of Canada (2016) reports that the OECD QSAR Toolbox (2012) profile suggests a high level of protein binding for this substance).

Brain concentrations were not significantly different to those in other tissues, but as it has the lowest lipid content, the lipid-normalized concentration in brain was the highest among all tissues. The study suggested transfer to and retention of Dechlorane Plus in chicken brain, despite its high molecular weight and hydrophobicity.

The f_{anti} values varied from 0.39 to 0.79 in chicken tissues. Fat (0.65), brain (0.64), and liver (0.64) had higher f_{anti} values than the other tissues (p < 0.01), as well as soil (0.52). The f_{anti} value for the whole chicken was calculated as 0.57 ± 0.07, which was significantly higher than those in soil (t-test, p = 0.048). However, the f_{anti} values ranged from 0.39 to 0.73 in chyme, intestinal contents and faeces, respectively, and these were not statistically significantly different, suggesting that gastrointestinal absorption was not stereoselective. It was estimated that soil contributed 94–100 % of the substance in chyme. These results suggest a preferential accumulation of the anti- isomer in chicken compared with soil, which is not due to differences in gastro-intestinal absorption efficiencies.

Anti-DP-1Cl was detected in most samples of atmospheric particulates and soil at concentrations of 2.2–7.5 and 16–51 µg/kg dw, respectively, but not in chicken food. It was detected in three chyme samples at concentrations from 0.07 to 0.96 µg/kg dw and one faeces sample (1.12 µg/kg dw). In chicken tissues, concentrations of anti-DP-1Cl were in the range of 'not detected' to 4.7 µg/kg ww. It was detected in 67 % of heart and 83.3 % of liver samples (the detection frequency in other tissues was below 34 %). Anti-DP-2Cl was not detected.

Significant correlation was found between anti-DP-1Cl and anti-DP ($p < 0.01$) in both atmospheric particulates ($r^2 = 0.98$) and soil samples ($r^2 = 0.79$), and in both liver ($r^2 = 0.99$, $p < 0.01$) and heart ($r^2 = 0.31$, $p < 0.05$).

The ratio of anti-DP-1Cl to anti-DP was lower in soil (0.005 ± 0.002) than atmospheric particulates (0.010 ± 0.003). The ratios in heart and liver varied from 0.0017 to 0.01 and 0.001 to 0.009, respectively. No significant differences were found in the ratios between soil and chicken tissues ($p > 0.05$), implying that anti-DP-1Cl mainly derives from the dietary uptake rather than *in vivo* dechlorination. This study is considered by to be reliable with restrictions, because the birds were not dosed in a standardised manner, and the variability in individual tissue concentrations was high. The validity of comparisons based on median concentrations only is therefore open to question. Since the birds appear to have been only around one year old at sacrifice, it is also possible that higher concentrations might have been achieved had the exposure duration been longer. However, the study is important because it shows that the anti- isomer is preferentially accumulated in chicken, which is presumably due to differences in metabolism and/or elimination. In addition, most trophic transfer studies in birds use measured concentrations in either liver or muscle only (see Section 5.5.1 of Appendix 1), and since these are not necessarily the main compartments for accumulation, whole body burdens might be significantly different.

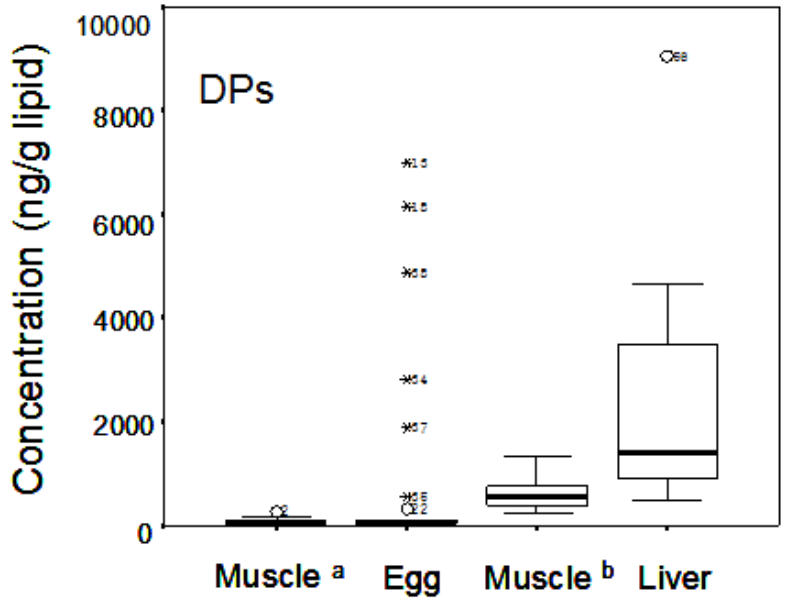
- Zheng *et al*. (2014b) investigated maternal transfer, potential metabolism and tissue distribution of Dechlorane Plus (and other flame retardants) during egg formation and embryo development in Domestic Chickens (*Gallus gallus domesticus*), apparently using the same birds as described in the study of Zheng *et al*. (2014a). Eggs were collected every day after the hens began laying, between March and April 2012 (n=79). The collected eggs were stored at ambient temperature in the yard. Nine chicks were hatched from ten collected eggs in an electric incubator and then sacrificed on the day of hatching, with liver and muscle tissues excised.[31] The hens were sacrificed in May 2012, and muscle samples (n=11) were collected. All samples were weighed and stored at -20 °C until further analysis, which followed the same procedures as Zheng *et al*. (2014a).

Dechlorane Plus (total isomers) was found in all of the samples, covering a wide range of concentrations from 0.77 to 9 034 µg/kg lw. The highest concentration was reported for a liver sample, but eggs also had a wide range of concentrations, some of which were around 7 000 µg/kg lw. The results are summarised in

Figure 4. The horizontal lines represents 5th, median and 95th percentiles, and the box represents 25th and 75th percentiles. Outliers exceeding 1.5 and 3 times of the height of box are shown as individual circles and asterisks, respectively. Unfortunately, the data for the two isomers and two potential transformation products are lumped together in the data table and figure in the original paper. Anti-DP-2Cl was not detected in any sample, but anti-DP-1Cl was found in 79 % of samples at concentrations between 'not detectable' and 30.7 µg/kg lw.

[31] Nine eggs that had not been incubated were also divided into albumin and yolk for analysis. However, no significant differences were found between levels in eggs from 0 d after the onset of incubation and these nine eggs (*t* test, $p > 0.05$) so their results are not provided in the paper.

Figure 4: Dechlorane Plus levels in chicken tissues



a: adult; b: chicken

(reprinted with permission from Zheng *et al*. (2014b). Copyright 2014: SETAC)

Eggs were analysed in three groups (collected at 0, 7 and 14 d of incubation, n=10 for each). No significant difference in concentration was found once extremes were removed (one-way ANOVA, $p>0.05$), with median levels of 16, 24 and 30 µg/kg lw, respectively. With the outliers included, the means (±standard error) were 1 336±878, 58±29 and 1 032±529 µg/kg lw (total concentration of Dechlorane Plus isomers and anti-DP-1Cl).

The mean (±standard error) concentration in hen muscle was 82±32 µg/kg lw (total concentration of Dechlorane Plus isomers and anti-DP-1Cl). The ratios of median concentrations in eggs to hen muscle for the syn- (0.40) and anti- isomer (0.67) were less than 1, indicating that these substances were more selectively retained by mothers. The maternal transfer potential of syn-Dechlorane Plus was lower than that of the anti- isomer. Consequently, the f_{anti} values in eggs (median 0.65, range 0.37 – 0.77; higher in albumin (0.89) than in yolk (0.59)) were significantly higher than those in hen muscle (median 0.56) ($p < 0.05$). The study therefore provides strong evidence for the stereoselective maternal transfer of Dechlorane Plus isomers to eggs.

The mean (±standard error) concentration in chick muscle and liver samples was 673±124 and 2 595±928 µg/kg lw, respectively (total concentration of Dechlorane Plus isomers and anti-DP-1Cl). The difference in levels in chick tissues compared with mid-incubation eggs was likely caused by lipid consumption during the last days of incubation[32]. Egg weights also decreased (by 10 % at 14 days after the onset of incubation) because of water loss and energy consumption during incubation.

With the assumption that the levels in eggs during incubation represented the initial level in eggs from which chicks were successfully hatched, the residual percentage of pollutants after incubation was roughly estimated by dividing the amount of substance in chicks (a summation of muscle and liver) by that in eggs (median levels were used for the calculation). The ratio for Dechlorane Plus was in the range 9 – 14 %. Although this estimation is crude

[32] The lipid contents in chick muscle and liver (6.3±1.7 % and 13.1±2.8 %, respectively) were significantly lower than that in eggs (14.3±2.9 %) ($p < 0.05$).

(uncertainty exists due to data heterogeneity and underestimations of whole-body amounts), the result suggests that there might have been significant metabolism by chicken embryos.

The f_{anti} values (0.68±0.02 in liver and 0.72±0.03 in muscle) in chick tissues were higher than those in eggs (0.65±0.07). The difference was statistically significantly different for muscle and eggs ($p < 0.01$). This suggests that stereoselective metabolism of the syn-isomer occurred during chicken embryo development.

This study is considered to be reliable with restrictions, because the birds were not dosed in a standardised manner, and the variability in individual tissue concentrations was high. The validity of comparisons based on median concentrations only is therefore open to question. However, the study shows that when exposure is sufficiently high, chicken eggs can accumulate up to around 7 mg/kg of Dechlorane Plus on a lipid weight basis [around 1 mg/kg ww[33]]. It also implies selective maternal transfer of the anti- isomer to eggs and stereoselective metabolism of the syn- isomer during chicken embryo development. In addition, it suggests that caution is needed in the interpretation of trophic transfer studies involving concentrations in bird eggs (see Sections 3.4.1.3 and 3.4.2.2), since they do not necessarily reflect parental whole body burdens.

Discussion

Studies with other highly hydrophobic substances such as decabromodiphenyl ether (ECHA, 2012a) suggest that achieving truly dissolved concentrations in many standard solvent vehicles is very difficult. The presence of undissolved microcrystals can mean that the degree of absorption across the gut can actually be higher at lower doses and solvent choice may also have an influence. This could confound conclusions drawn from any apparent dose-response relationship, since the nominal concentrations might not represent the actual dissolved concentrations. Dechlorane Plus does seem to have a reasonable degree of solubility in several organic solvents (see Section 1.5), so the variation seen might perhaps be more related to saturation of various pathways at different doses. The studies by Zheng *et al*. (2014a&b) involved birds contaminated from their local environment, so at least represent concentrations that can actually be achieved in nature.

The main conclusions that can be drawn from these bird studies is that uptake, distribution and elimination kinetics are complex. Zheng *et al*. (2014a) detected the substance in all tissues examined in a sample of Domestic Chickens (*Gallus gallus domesticus*): concentrations were variable, but the data suggest that concentrations were typically highest in fat (median: 10 µg/kg ww), with liver (median: 4.4 µg/kg ww) and muscle (median: 0.92 µg/kg ww) having lower concentrations. Fat was the most important reservoir (accounting for 56 % of the residues), followed by liver (12 %) and gonad (12 %). Muscle contained significantly lower concentrations than other tissues ($p < 0.05$). The comparable concentrations in these other tissues suggests that lipid content was not the only factor that influenced tissue distribution, and it was speculated that blood perfusion and sequestration by proteins could be additional factors. Wet weight brain concentrations were not significantly different to those in other tissues, but as it has the lowest lipid content, the lipid-normalized concentration in brain was the highest among all tissues. There was some evidence of preferential accumulation of the anti- isomer in chicken compared with soil. In contrast, the Zheng *et al*. (2014b) study found that liver had the highest levels in chicks of this species (up to 9 034 µg/kg lw). Similarly, Li *et al*. (2013a) found the highest concentrations in liver in Common Quail *Coturnix coturnix* (~1 750 mg/kg lw in the 10 mg/kg/d dose group after 90 days; lower total levels were found at the 100 mg/kg/d dose group, and there appeared to be enrichment of the syn- isomer as exposure increased).

Like fish, residues increased with longer exposure times and a steady state was not achieved. It is not known how long it would take to achieve a steady state in chicken or quail, and none of the studies investigated depuration kinetics.

[33] The lipid content of the eggs was 15 ± 4, 14 ± 2 and 15 ± 4 per cent on days 0, 7 and 14 of incubation, respectively.

Zheng *et al*. (2014b) reported a wide range of concentrations in Domestic Chicken eggs and chicks. Concentrations in the mother birds were higher than in the eggs and chicks, and the maternal transfer potential of the syn- isomer was lower than that of the anti- isomer. There could also have been stereoselective metabolism of the syn- isomer during chicken embryo development. The study also showed that when natural exposure is sufficiently high, birds' eggs can accumulate up to around 7 mg/kg of Dechlorane Plus on a lipid weight basis [around 1 mg/kg ww].

3.4.2.3 Field studies

Six biota monitoring studies that report some measure of bioaccumulation potential have been summarised in detail in Appendix 1 (Sun *et al*., 2012; Yu *et al*., 2013; Barón *et al*., 2014a; Yu *et al*., 2014; Muir *et al.,* 2014 [ABST]; Peng *et al.*, 2015). Other terrestrial wildlife monitoring results from the open literature are reported in Appendix 5.

Monitoring studies for terrestrial wildlife face the same problems and limitations as those involving aquatic species (see Section 3.4.1.3). The available data show that European terrestrial wildlife is contaminated with Dechlorane Plus, so uptake is clearly occurring. Many European samples have been collected from relatively unpolluted environments, where the substance is more likely to be present due to long range transport rather than (in)direct local releases. Levels therefore tend to be lower than in more polluted environments close to the U.S. manufacturing site and in China (e.g. by two orders of magnitude in the study of Guerra *et al*., 2011).

The highest concentration in terrestrial bird eggs reported to date is around 7 mg/kg lw [around 1 mg/kg ww] (Zheng *et al*., 2014b) for Domestic Chicken (*Gallus gallus domesticus*) from an e-waste recycling region in southern China (Zheng *et al*. (2012) reported a slightly lower maximum concentration of just over 3 mg/kg lw). Sun *et al*. (2012) and Chen *et al*. (2013b) found that other terrestrial bird tissues (e.g. liver and muscle) may accumulate Dechlorane Plus up to 3 820 µg/kg [3.82 mg/kg] lw (approximately 500 µg/kg [0.5 mg/kg] ww). In addition, the latter study suggests that Dechlorane Plus burdens in terrestrial organisms could be driven by the accumulation of the anti- isomer, and factors other than lipid solubility (e.g. hepatic binding enzymes) could play important roles in determining deposition in terrestrial bird tissues (based on the ratio of muscle and liver levels). Similar findings apply to other highly hydrophobic halogenated substances, such as decabromodiphenyl ether (ECHA, 2012a).

Four studies have attempted to investigate biomagnification potential in terrestrial food chains involving birds:

- Yu *et al*. (2013) investigated the biomagnification of Dechlorane Plus in two terrestrial food chains in China. BMFs based on median lipid normalized concentrations were 2 in a rat–owl food chain, indicating biomagnification, and 0.3 for a sparrow–kestrel food chain, indicating biodilution. As previously noted, lipid normalisation might not be appropriate for this substance, and when wet weight is considered, the BMFs become 0.9 and 0.4, respectively. No significant differences were found for the BMF values of the two isomers in either feeding relationship. Other studies have found significantly higher concentrations of Dechlorane Plus in some of the same species from the same general area, which raises some doubts about the reliability of the BMFs, although they could be higher.

- Barón *et al*. (2014a) investigated biomagnification potential for terrestrial birds of prey in a Spanish national park using egg concentrations (total isomers) and stable isotope analysis. Although the paper did not find a clear linear correlation with trophic position, a strongly positive trend would result if the species in the highest trophic position is discounted (only a single egg was collected for that species).

- Sun *et al*. (2012) found a positive correlation between log normalized median muscle concentrations (syn- and anti- isomer separately) and $\delta^{15}N$ values at both rural and

urban sites for three terrestrial passerine bird species: Light-vented Bulbul (*Pycnonotus sinensis*), Long-tailed Shrike (*Lanius schach*), and Oriental Magpie-robin (*Copsychus saularis*) collected from southern China. Whilst this could imply biomagnification, comparisons of trophic position between locations based on raw $\delta^{15}N$ data are inappropriate since the baseline $\delta^{15}N$ values for each site are unknown. It is also unclear how the calculations would be affected if the concentrations were expressed as a geometric mean rather than median concentration. The variation in trophic status is also surprisingly wide for each species. The actual level of accumulation from the diet cannot be assessed. This study therefore only provides equivocal evidence that contamination levels increase with trophic position in terrestrial passerines.

- Peng *et al.* (2015) investigated the accumulation of Dechlorane Plus in terrestrial passerines from a national nature reserve in South China. The levels of total Dechlorane Plus ranged from 1.2 to 104 µg/kg lipid and the levels were found to be significantly (p=0.03) higher in insectivorous birds (mean 16.9 µg/kg lipid; n-=27) than in omnivorous birds (mean 6.4 µg/kg lipid; n-17). As insectivorous birds generally occupy a higher trophic level than omnivorous birds Peng *et al*. (2015) considered that this could be an indication of biomagnification. Unfortunately the trophic levels of the birds in the study were not determined.

The interpretation of trophic transfer studies involving terrestrial birds is complicated by the fact that sample sizes are small, and a single tissue (usually egg, muscle or liver) is typically analysed. Zheng *et al*. (2014a) showed that fat tissue is an important compartment for Dechlorane Plus accumulation in the Domestic Chicken (*Gallus gallus*), so whole body burdens might be significantly different from those implied by measured concentrations in a single tissue (particularly muscle). Coupled with the uncertainties about the relevance of lipid normalisation, firm conclusions about trophic transfer in birds cannot be drawn from the available data.

Guerra *et al*. (2011) found that the concentration of total Dechlorane Plus isomers in Peregrine Falcon *Falco peregrinus* eggs from birds with a known terrestrial diet was slightly lower than those with an aquatic-based diet in Spain, but of a similar order of magnitude in Canada. This study suggested differences in uptake in the two isomers, with the relative concentration of the anti- isomer in eggs from birds with a known terrestrial diet being significantly higher in Spanish samples compared to their Canadian counterparts.

Detection of Dechlorane Plus and anti-[DP-1Cl] in birds' eggs shows that maternal uptake and transfer to eggs can occur. Similarly, detection of these substances in human cord serum indicates transfer across the placenta and exposure of foetuses. Presence in human breast milk also means that exposure can continue prior to weaning. Sensitive life stages are therefore exposed to the substance. Concentrations in maternal sera imply that elevated concentrations may arise following prolonged exposure.

In addition, a number of studies have examined contamination of human tissues (Ren *et al*. (2009); Yan *et al*. (2012); Ben *et al*. (2013); Ben *et al*. (2014); Zhang *et al*. (2013); He *et al*. (2013); Wang *et al*. (2014); Chen *et al*. (2015); Cequier *et al*. (2013); Sahlström *et al*. (2014); Brasseur *et al*. (2014); Siddique *et al*. (2012); and Zhou *et al*. (2014)) and these are briefly summarised in Appendix 1. Studies involving humans are complicated by exposure in occupational and consumer settings. It is therefore difficult to interpret these data in terms of bioaccumulation assessment. Most of the studies are from China, and reflect local sources that presumably include exposure to Dechlorane Plus as vapour or in/on particulates at higher levels than might be expected under 'normal' environmental conditions (including exposure to contaminated indoor dust). However, the substance has also been detected in breast milk samples from North America, and is present in blood serum in the European population. The distribution of concentrations in various tissues is often skewed, with some high values but many lower ones. However, the studies clearly show that Dechlorane Plus is bioavailable to humans and frequently detected in general populations (with higher levels in workers). The Zhang *et al*. (2013) study is interesting because it showed an increase in blood levels with presumed exposure time (working life). Exposure to developing foetuses and new born children can occur due to its presence in placenta and breast milk. Concentrations in non-occupationally exposed people

appear to be typically in the region of 1-10 µg/kg lw depending on the tissue. The highest reported concentration in human blood is around 3 000 µg/kg [3 mg/kg] lw (Zhang *et al*., 2013), although given the low lipid content of blood, the wet weight concentration would be much lower. Anti-DP-1Cl is also frequently detected at lower concentrations.

In addition, Dechlorane Plus has been detected in terrestrial plants. For example, Wang *et al*. (2013a) detected mean total isomer concentrations of 1 038 µg/kg ww for vegetables and 877 µg/kg ww for grains collected at a site close to the Chinese manufacturing facility[34]. The maximum (for one vegetable type) was 2 720 µg/kg [2.72 mg/kg] ww.

3.4.4 Summary and discussion of bioaccumulation

Dechlorane Plus is very hydrophobic, but its molecular dimensions and relatively high n-octanol solubility indicate potential for bioaccumulation. Uncertainties in the physico-chemical data set make QSAR predictions based on log K_{OW} difficult to interpret, but a fish BCF of ≤5 500 L/kg is suggested. Aqueous exposure is likely to be of limited importance in terms of bioaccumulation potential. There are no reliable measured fish BCF data. Based on the apparent water solubility limit and reported concentrations in fish in the available aquatic exposure studies, BCFs above 10 000 L/kg can be estimated, but these are confounded by likely oral ingestion of the substance as a precipitate and/or adsorbed to food. The maximum wet weight fish concentrations measured in the aqueous BCF studies were 0.385 – 8.72 mg/kg after 30 days for Bluegill Sunfish *L. macrochirus* and 0.327 mg/kg after 56 days for Common Carp *C. carpio*, although this does not necessarily represent the concentration at steady state (it might also include the substance adsorbed to skin or present in the gut).

Dietary studies provide a more environmentally realistic exposure route for very hydrophobic substances. Whilst none of the available laboratory studies suggest a BMF > 1, they indicate that that it takes a long time to reach steady-state conditions (beyond 50 days), and that Dechlorane Plus has a long depuration half-life (around 50 days or more in fish). Tomy *et al*. (2008) is the best dietary study available, but the raw data are not available for analysis. Based on correlations and theoretical considerations, the long depuration half-lives for Dechlorane Plus observed in fish by Tomy *et al*. (2008) (supported by the findings of Zeng *et al*., 2014a) are considered highly indicative that the fish BCF could exceed 5 000 L/kg. Other dietary studies show that juvenile *O. mykiss* can absorb at least 16 % of the dose from a single feed, and the substance has been shown to distribute throughout the body in *C. carpio*, including gonadal tissue.

Studies with mammals and birds show complex uptake, distribution and elimination kinetics with preferential accumulation in liver rather than muscle or blood in some species. The substance can be found in gonads and brain tissue, and can be transferred from mothers to eggs. There are similar findings in amphibians (Li *et al.*, 2014b). The comparable concentrations in most tissues suggests that lipid content may not be the only factor that influences tissue distribution; blood perfusion and sequestration by proteins could be additional factors. Like fish, residues in birds and mammals appear to increase with longer exposure times and steady state was not achieved in the laboratory tests. Also like fish, Dechlorane Plus has a long elimination half-life from rat liver, in the region of 180 days or more (Li *et al*. (2013b), discussed in Section 4.1).

There is conflicting information available on BSAFs although they can be difficult to interpret for highly hydrophobic substances. Four studies suggest BSAFs below 1 and one laboratory study also gave BSAF values below 1 for *Lumbriculus variegatus*. However, one field study reported BSAFs above 1 for three fish species, with values up to 3.2 and 9.0 for the anti- and syn- isomers, respectively and another reported BSAFs above 2 for molluscs. A BSAF above 2 suggests that the substance can accumulate to a greater extent than by simple partitioning alone. For two

[34] Wang *et al*. (2010) reported soil concentrations in the range 377 – 13,400 µg/kg dw from sampling sites within 0.5 km of the Chinese manufacturing site (decreasing by an order of magnitude within 7.5 km).

studies where the BSAF was below 1, the BSAF values for Dechlorane Plus were similar to those for BDE-183 (a heptabromodiphenyl ether) in the same samples.

Several field studies have attempted to measure biomagnification between trophic levels, in both aquatic and terrestrial environments. Some studies suggest trophic dilution, whereas others suggest trophic magnification. The lack of a standardised approach to the assessment of trophic magnification and other confounding factors (including variable exposure across concentration gradients in the sampled environment, small sample sizes, analysis of single tissues, and reliance on specific feeding relationships) mean that none of the studies is fully reliable.

Monitoring studies show that the two isomers of Dechlorane Plus are widely dispersed in the European aquatic and terrestrial environment. Evidence from around the world shows that uptake occurs in a wide range of wildlife species throughout freshwater and marine aquatic food webs, from invertebrates and fish to piscivorous top predators such as Bald Eagle *Haliaeetus leucocephalus*, Double-crested Cormorant *Phalacrocorax auritus*, Eurasian Otter *Lutra lutra* and cetaceans (e.g. *Delphinus delphis*). Dechlorane Plus is also found in bird- and mammal-eating predators associated with both aquatic and terrestrial environments (such as Peregrine Falcon *Falco peregrinus* and Polar Bear *Ursus maritimus*). From the small number of studies available, maximum levels in top predators in Europe are broadly in the range 0.1 – 1 µg/kg ww, although there are many non-detects.

Many European samples have been collected from relatively unpolluted environments, where the substance is more likely to be present due to long range transport rather than (in)direct local releases. Such studies show that Dechlorane Plus can be present in wildlife (including top predators) in regions relatively remote from human activity, although the levels are typically very low (<0.1 µg/kg ww). In contrast, studies in more polluted environments in China and North America show higher levels, indicating that organisms have the potential to accumulate in the region of 1 – 10 µg/kg ww. The highest concentrations have been observed in China, at up to 1 mg/kg ww [97 mg/kg lw] in fish muscle in the vicinity of point sources (whole body concentrations are not available), and around 0.5 – 1 mg/kg ww [3 – 7 mg/kg lw] in terrestrial bird tissues, including up to around 7 mg/kg lw [~1 mg/kg ww] in birds' eggs. This suggests that if exposure in the European environment were to increase, levels in wildlife might also increase. The higher levels that have been found in wildlife exceed those that are considered to be of concern within a bioaccumulation context (e.g. a "critical concentration" of 0.065 or 0.65 mg/kg ww [1.3 or 13 mg/kg lw] can be estimated for Dechlorane Plus based on a critical body burden of 0.001 mmol/kg ww recommended in the REACH PBT Guidance (ECHA, 2017a); see Appendix 7).

Differences in bioaccumulation patterns in terrestrial environments compared to aquatic are difficult to distinguish based on the available information. The concentration of total Dechlorane Plus isomers in Peregrine Falcon *Falco peregrinus* eggs from birds with a known terrestrial diet was slightly lower than those with an aquatic-based diet in Spain, but of a similar order of magnitude in Canada. Dechlorane Plus has also been detected in terrestrial plants at mean concentrations of 1 mg/kg ww for vegetables and 0.9 mg/kg ww for grains collected at a site close to the Chinese manufacturing facility (with a maximum for one vegetable type of 2.72 mg/kg ww). Uptake and translocation in plants has also been shown in a laboratory study although the results may have been affected by toxicity caused by other substances.

Although not easily interpretable in the context of bioaccumulation potential, studies of human exposure also suggest an increase in blood levels with presumed exposure time (working life). Concentrations in non-occupationally exposed people appear to be typically in the region of 1-10 µg/kg lw depending on the tissue. The highest reported concentration in human blood is around 3 000 µg/kg [3 mg/kg] lw, although given the low lipid content of blood, the wet weight concentration would be much lower. Detection in human cord serum indicates transfer across the placenta and exposure of human foetuses, and presence in human breast milk also means that exposure can continue prior to weaning.

The amount of information available for the anti- and syn- isomers varies amongst the studies. Where the information is available, the syn- isomer appears to be somewhat more

bioaccumulative as it reaches slightly higher body concentrations or has a longer depuration half-life. However, the results for the anti- isomer remain of concern. For instance, the depuration half-life in the key fish bioaccumulation study is around three times the bench-marked value for other vB substances. Without a better understanding of what might affect bioaccumulation behaviour of the two isomers (for example metabolic pathways and/or switches between isomers *in vivo*), the two isomers are assumed to have the same bioaccumulation potential in the absence of further information.

4 Human health hazard assessment

Mammalian toxicity data are summarised in detail in Appendix 1. They are not included in the main part of this report because the proposal is to identify Dechlorane Plus as vPvB only. This section includes relevant information for the bioaccumulation assessment.

4.1 Toxicokinetics (absorption, metabolism, distribution and elimination)

A toxicokinetic study conforming to OECD TG 417 is not available.

The registration dossier includes two robust study summaries for toxicokinetics assessment:

- The first unreferenced study, considered reliable with restrictions by the Registrant (due to the small number of animals) was performed in 1983. No guideline was followed and the study was not subject to GLP. ^{14}C-Radiolabelled Dechlorane Plus (31.5 mCi/mmol) was administered in corn oil once by oral gavage at a dose of 1 mg/kg bw (corresponding to 4.8 µCi) or 113 mg/kg bw (corresponding to 3.8 µCi) to Sprague-Dawley rats (*Rattus norvegicus*). The composition of the substance was stated to be the same as the commercial substance (the ratio of isomers was 5.4:1). The low dose group consisted of three females and two males, and the high dose group consisted of two females. Another group of two females was fed non-labelled Dechlorane Plus at 1 % in the diet for 14 days before gavage administration. No control animals were used. Excretion via urine, faeces, and expired air and residual concentrations in organs and carcass were determined.

One rat was used for monitoring radioactivity in expired air and one rat was used for monitoring the time course of blood levels for 48 hours after administration of 1 mg/kg bw. Urine and faeces were collected from all remaining rats for 4 days, then the animals were killed and radioactivity in 17 different organs/tissues and carcass were determined.

At four days after a single oral administration of 1 mg/kg bw, the percentage of the dose excreted in faeces was 83.5 % for females and 92.7 % for males (0.07 % and 0.01 % in urine, respectively). This indicates a maximum absorption of 16.5 % in females and 7.3 % in males.

The percentage of the dose excreted in faeces in the higher dose (113 mg/kg bw) group was 96.5 % for females (0.009 % in urine). This indicates a maximum absorption of 3.5 % in females.

Four days after single oral administration of 1 mg/kg bw to rats pre-treated with the non-labelled substance at 1 % in diet for 14 days, 102 % of the dose was excreted in faeces (0.03 % in urine) indicating almost no absorption.

Excretion in expired air amounted to 0.004 % of the administered dose within 4 days.

The concentrations in all organs and tissues investigated, besides liver and residual carcass, were below 1 ppm. At the high dose, the liver of females contained 1.66 ppm and the residual carcass contained 1.25 ppm. All organs and tissues besides liver and residual carcass contained well below 1 % of the dose. The livers of males and females at the low

dose contained 1.60 and 2.29 % of the dose, respectively. The residual carcass of males and females at the low dose contained 5.09 and 5.05 % of the dose, respectively. The residual carcass of females at the high dose contained 0.90 % of the dose. The carcass of pre-treated females contained 4.44 % of the dose.

Metabolites were not investigated.

The Registrant concludes that "almost no" absorption occurs after oral administration to a single dose.

- The second unreferenced study, considered reliable with restrictions by the Registrant (due to the small number of animals and detection of more than 100 % of the administered dose in the faeces) was performed in 1979. No guideline was followed and the study was not subject to GLP. Two groups of three Sprague-Dawley rats (*R. norvegicus*) each were given a single oral dose of 0.57 mg of ^{14}C-radiolabeled Dechlorane Plus (27.6 µCi in 0.5 mL) (purity stated to be the same as the commercial substance) suspended in water with 5 % Tween 80 and 5 % gum arabic. Three rats were killed 4 hours after administration and the remainder after 24 hours, and Dechlorane Plus was determined by liquid scintillation in blood, kidneys, liver, urine, and faeces.

Within 4 hours after oral administration, less than 0.1 % was excreted in urine. At 24 hours, less than 1 % had been excreted in urine, and a mean of 94.6 % (range: 76.1 – 104.8 %) of the administered dose was excreted in faeces, indicating a maximum absorption of around 5.4 %. The sum of total radioactivity detected in blood, kidneys, liver, and urine was at or below 6 % of the total dose.

Blood (total blood volume) contained less than 2 %, kidneys contained less than 1 %, and liver contained less than 5 % of the administered dose.

One metabolite was found in the liver but its identity and concentration was not reported.

The Registrant concludes that Dechlorane Plus is poorly absorbed after oral administration, at a maximum of 6 % of the administered dose. Highest concentrations are found in the liver.

- A third study missing from the registration dossier is Li *et al*. (2013b), who exposed male Sprague–Dawley rats (*R. norvegicus*) (35 days' old with an average weight of 110 g) to commercial Dechlorane Plus mixed in corn oil by oral gavage for 90 days at different doses (0, 1, 10, and 100 mg/kg/d). Another group was exposed to 100 mg/kg/d of the substance for 45 days followed by 45 days' depuration, together with a control group that was fed uncontaminated food. Forty-two animals were used in all. Liver, muscle and serum samples were analysed using GC-MS. The LoDs for the anti- and syn- isomers were 70.44 and 108.31 µg/kg lw in muscle/liver, and 0.054 and 0.20 ng/mL in serum, respectively.

Both isomers were detected in all of the tissues measured in the control group at the end of the 90-day exposure period (average concentrations in liver were 2.8 ± 1.2 and 0.9 ± 0.4 mg/kg lw for the anti- and syn- isomer, respectively according to the paper, but the supplementary data give slightly different values), indicating a background level of exposure (e.g. from feed or air-borne dust). DP-1Cl and DP-2Cl were not detected in the control group (LoDs for anti-DP-1Cl were 0.98 µg/kg lw in muscle and liver, and 0.042 ng/mL in serum, respectively).

In the exposure groups, the highest concentrations of both isomers were detected in liver from the 100 mg/kg/d dose group, at 320 ± 49 mg/kg lw for the anti- isomer and 750 ± 120 mg/kg lw for the syn- isomer (i.e. total concentration ~1 000 mg/kg lw). These levels were 12–15 times higher than in muscle and around 5 times higher than in serum from the same group. In liver, the concentration of both isomers increased with the dose. However, in muscle, the highest concentration of the syn- isomer occurred in the 10 mg/kg/d group (~85 mg/kg lw). Some of the statements in the paper about highest concentrations of the

anti- isomer and in other tissues do not seem to match the information provided in the supplementary data.

The concentration ratio of the anti- isomer to total isomers was similar to the commercial substance in the 1 mg/kg/d group, but significantly decreased in the two higher dose groups, suggesting enrichment of the syn- isomer with increasing dose.

The highest average concentration of syn-DP-1Cl and anti-DP-1Cl occurred in liver in the 100 mg/kg/d group, reaching 140 ± 51 and 480 ± 170 µg/kg lw, respectively. Average concentrations were 5-8 times lower in muscle in the same group, but whereas levels in liver increased with dose, levels in muscle remained fairly constant. Two additional unidentified substances were detected in liver as well as in the commercial substance.

The treatment group that was exposed to 100 mg/kg/d for 45 days accumulated a lower amount of total isomers in the liver compared to the animals exposed for 90 days (achieving an average total liver concentration of ~310 mg/kg lw). This suggests that residues increase with longer exposure times. The amounts of both isomers in muscle and liver showed no statistically significant change during depuration, although levels in serum decreased significantly. The content ratio of syn- and anti- isomers in liver to those in liver plus muscle significantly increased after depuration compared with the end of the uptake phase. These data suggest that Dechlorane Plus is more prone to accumulate in liver or that the elimination rate in liver is lower than that in muscle. The content of both syn- and anti-DP-1Cl in the liver decreased significantly after depuration and neither was detected in serum after depuration. The elimination half-life of the syn- isomer was about 179 days in liver, 44 days in muscle and 24 days in serum. The elimination half-life of the anti- isomer was 54 days in muscle and 25 days in serum (the figure for liver is not provided as the concentration increased during the depuration phase, although not significantly).

In conclusion, both isomers preferentially accumulate in liver rather than muscle or blood, and have a long elimination half-life in rats. Residues appear to increase with longer exposure times – it is not known how long it would take to achieve a steady state.

Discussion

Based on the two studies summarised in the registration dossier, a single oral dose of 1 mg/kg bw in rats may lead to a maximum absorption of between 5 and 20 %. Higher doses, or dosing following 14 days' prior exposure suggest a lower level of absorption, although if the substance was present as microcrystals in the vehicle, the nominal concentrations might not reflect actual exposure to dissolved substance. About 90 % of the substance is excreted unchanged in faeces (excretion in urine and expired air is below 0.1 %). The absorbed substance is widely distributed in the body, with the highest concentration in liver. Four days after administration, between 1 % and 10 % of the administered dose was found in the carcass. Levels of around 1 – 2 ppm [mg/kg] may be reached in both liver and the residual carcass.

The main conclusion that can be drawn from the Li *et al*. (2013b) study is that uptake, distribution and elimination kinetics are complex, with preferential accumulation in liver rather than muscle or blood. Dechlorane Plus achieved levels of ~1 000 mg/kg lw in rat liver when dosed at 100 mg/kg/d via oral gavage for 90 days (the paper does not provide the lipid content so a wet weight concentration cannot be estimated). Like fish, residues increased with longer exposure times and a steady state was not achieved. Also like fish, Dechlorane Plus has a long elimination half-life from rat liver, in the region of 180 days or more.

None of the studies permit firm conclusions to be drawn about the level of accumulation or tissue distribution following exposure to low concentrations over long time periods.

5 Environmental hazard assessment

Aquatic and terrestrial organism toxicity data are summarised in detail in Appendix 1. They are not included in the main part of this report because the proposal is to identify Dechlorane Plus as vPvB only.

6 Conclusions on the SVHC Properties

6.1 CMR assessment

Not relevant for the identification of the substance as SVHC in accordance with Article 57(e) of REACH.

6.2 PBT and vPvB assessment

6.2.1 Assessment of PBT/vPvB properties

A weight-of-evidence determination according to the provisions of Annex XIII of REACH is used to identify the substance as vPvB. All available information (such as the results of standard tests, monitoring and modelling, information from the application of the analogue approach (read-across) and (Q)SAR results) was considered together in a weight-of-evidence approach.

6.2.1.1 Persistence

The available data on persistence have been considered in terms of unequivocal, equivocal and negative evidence that the substance is very persistent.

Unequivocal evidence

- There are no measured half-life data for degradation of Dechlorane Plus in surface water, sediment or soil. Therefore there is no unequivocal evidence that the half-life of the substance in these media is sufficiently long to meet the Annex XIII criteria for P or vP.

Equivocal evidence

- Predictions using the BIOWIN model suggest that biodegradation of Dechlorane Plus will be very slow. The BIOWIN results are the same as a number of hexachloro-norbornene-containing analogue chemicals that have been agreed to be persistent organic pollutants (POPs) under the Stockholm Convention. These POPs are considered to have long environmental half-lives consistent with the vP criteria of Annex XIII. For example, heptachlor has a half-life of up to two years in soil, whereas the half-life of chlordane in soil is around one year. These two substances are the closest analogues to Dechlorane Plus with measured data. Soil and sediment simulation studies for endosulfan (a much more polar substance) indicate that the hexachloro-norbornene part of the molecule is very stable: the water-sediment half-life was >120 days and the soil half-life of metabolites was between 123 and 391 days.
  In addition, the two possible microbial degradation pathways predicted for Dechlorane Plus are the same as the POP analogues. It is unlikely that metabolic rates for these pathways will be more rapid for Dechlorane Plus, which is significantly less water soluble than the POPs.

  This information is assigned a high weighting in the persistence evaluation.

- The water solubility data for Dechlorane Plus (<2 ng/L), together with a likely log K_{OW} value above 9, suggests that it will be strongly bound to organic carbon in sediment/soil particles and therefore likely to be of low bioavailability to micro-organisms for biodegradation. In addition, studies with fish do not suggest a high potential for biotransformation (e.g. Tomy *et al.*, 2008). This supports the premise that the molecule is metabolically recalcitrant.
- Dechlorane Plus was detected in a lake sediment core layer corresponding to around 1980

(Qiu *et al*., 2007). Whilst this does not provide any information about half-life, it shows that the substance can persist in sediments for over 20 years.

- Dechlorane Plus has been detected in remote locations such as the Arctic and Antarctic in air, sediment and in wildlife such as Arctic Char (*Salvelinus alpinus*), Glaucous Gull (*Larus hyperboreus*), Black Guillemot (*Cepphus grylle*), Common Guillemot (*Uria aalge*), Ringed Seal (*Phoca hispida*) and Polar Bear (*Ursus maritimus*). This indicates long range transport of Dechlorane Plus, probably as a result of adsorption to particulates in the atmosphere.

Negative evidence

- None of the available data clearly show that Dechlorane Plus is rapidly degraded in water, sediment or soil. One biodegradation screening study (Chou *et al*., 1979) reports low recoveries of radioactivity under aerobic conditions after six weeks' incubation. However, as discussed in Section 3.1.2.1.2 of Appendix 1, there are significant deficiencies in the methodology. It was not performed according to a standard test guideline and the analytical method was inappropriate to assess the degradation of such a poorly water soluble substance as Dechlorane Plus. It is not considered to be valid.

In summary, although there are no environmental simulation studies available for Dechlorane Plus that provide an unequivocal measurement of the half-life in water, sediment or soil, several lines of evidence indicate that it meets the vP criteria[35] in Annex XIII of REACH. In particular, the predicted low level of biodegradability and structural similarity to chemicals that have been agreed to meet the Stockholm Convention definition of a POP, and lack of any predicted degradation pathways that are different to the POPs strongly point to the high probability that it will not degrade any faster than them. This conclusion is supported by its very low water solubility (suggesting limited bioavailability to micro-organisms once bound to solid matrices), lack of evidence of biotransformation in fish (supporting the premise that the molecule is metabolically recalcitrant) and field evidence suggesting persistence in sediment as well as widespread presence in remote regions.

6.2.1.2 Bioaccumulation

The available data on bioaccumulation have been considered in terms of unequivocal, equivocal and negative evidence that the substance is very bioaccumulative.

Unequivocal evidence

- None of the available BCF studies is valid, so it is not possible to unequivocally demonstrate that the substance has a BCF value above 2 000 L/kg (B) or 5 000 L/kg (vB). Based on the apparent water solubility limit and reported fish concentrations in the available aquatic exposure studies, BCFs above 10 000 L/kg can be estimated, but these are confounded by likely oral ingestion of the substance as a precipitate and/or adsorbed to food. They are therefore assigned a low weighting for the purposes of this analysis. Dietary exposure is more relevant, so the lack of a valid BCF study is not a hindrance for the evaluation of bioaccumulation.

[35] A substance fulfils the vP criterion when the half-life is higher than:

- 60 days in marine, fresh- or estuarine water, or
- 180 days in marine, fresh- or estuarine water sediment, or
- 180 days in soil.

These are the same as the Stockholm Convention criteria.

Equivocal evidence

- Dechlorane Plus is a very hydrophobic substance, with a measured solubility in pure water below 2 ng/L (0.002 µg/L) at 20 °C and estimated log K_{OW} value ≥9. This meets the screening vB criterion (log K_{OW} ≥5). QSAR predictions based on log K_{OW} are uncertain, but a fish BCF of up to 5 500 L/kg is suggested, and one model predicts a BAF of 7.5×10^5 L/kg ww. Its molecular weight of 654 g/mole, estimated maximum diameter of around 1.4 nm and n-octanol solubility of 470 mg/L at 25 °C mean that significant uptake via passive transport cannot be ruled out if aquatic organisms are exposed. Indeed, the n-octanol solubility suggests that lipid solubility might be relatively high, and fat tissue was the most important reservoir for the substance in a study involving Domestic Chickens (Zheng *et al*., 2011a). In addition, several studies suggest that factors other than lipid solubility (e.g. hepatic binding enzymes, blood perfusion and sequestration by proteins) could play important roles in determining tissue deposition (e.g. Zeng *et al*. (2014a), de la Torre *et al*. (2012), Guerra *et al*. (2011), Zhang *et al*. (2011a) and Li *et al*. (2013a&b) and Chen *et al*. (2013b)). The log K_{OA} >5 also suggests that it has a high biomagnification potential in terrestrial wildlife.
- The key dietary bioaccumulation study of Tomy *et al*. (2008) with juvenile Rainbow Trout *O. mykiss* is reliable with restrictions**.** In the absence of the original raw data, a definitive conclusion about the BMF from this study cannot be drawn. The theoretical BMF could have been around 0.12 for the syn- isomer. A BMF of 0.1 or above correlates with a lipid-normalised BCF of 2 000 L/kg based on both theoretical arguments and empirical observations (MacKay *et al*., 2013 and Inoue *et al.*, 2012).
- With one exception (musk xylene), substances already agreed to meet the vB criterion or identified as POPs based on BCF values generally have relatively long depuration half-lives of 20 days or more in at least one fish species (see Appendix 6 and 7). A depuration half-life above around 8-10 days is also suggestive of a lipid-normalised and growth-corrected BCF above 5 000 L/kg according to the analysis in EA (2012). The depuration half-life for Dechlorane Plus was around 30-40 days for the anti- isomer and 50-70 days for the syn-isomer in the dietary study of Tomy *et al*. (2008) with juvenile Rainbow Trout *O. mykiss*.[36] Zeng *et al*. (2014a) found that residues remained in all tissues in a study using Common Carp *C. carpio* after 45 days of depuration. The long depuration half-life for Dechlorane Plus in fish is therefore highly indicative of a very bioaccumulative substance. Dechlorane Plus also has an elimination half-life from liver in the region of 180 days or more in Brown Rats *Rattus norvegicus* (Li *et al*., 2013b). These findings are assigned a high weight in the bioaccumulation assessment.
- Dechlorane Plus is bioavailable in laboratory studies. For example, juvenile *O. mykiss* can absorb at least 16 % of the dose from a single feed (Xiao *et al*., 2013). All available dietary laboratory studies suggest that it takes a long time to reach steady-state. Monitoring studies show that the two isomers of Dechlorane Plus are widely dispersed in aquatic and terrestrial food webs, including in areas relatively remote from human activity such as the Arctic and Antarctic. This involves invertebrates and fish as well as predatory birds and mammals such as Double-crested Cormorant *Phalacrocorax auritus*, Bald Eagle *Haliaeetus leucocephalus*, Peregrine Falcon *Falco peregrinus*, Eurasian Otter *Lutra*, Wolf *Canis lupus*, Polar Bear *Ursus maritimus* and cetaceans (e.g. *Delphinus delphis*). The highest levels are observed in polluted environments in China and near the U.S. manufacturing facility.
- Wang *et al*. (2015) reported field BSAFs above 1 for three fish species, with values up to 3.2 and 9.0 for the anti- and syn- isomers, respectively and Jia *et al*. (2011) reported BSAFs above 2 for molluscs. A BSAF above 2 suggests that the substance can accumulate to a greater extent than by simple partitioning alone.
- There is some field evidence of biomagnification between trophic levels:

[36] Arnot & Quinn (2015) estimated a growth-corrected depuration half-life of about 100 days for a ca. 15 g fish with 5 % lipid content using the results of the Zitko (1980) study with Atlantic Salmon *S. salar*. However, this study is not valid for a variety of reasons: in particular co-exposure to other related substances might have affected the uptake or metabolism of Dechlorane Plus, as well as the health of the fish.

i) Wu *et al*. (2010) calculated a TMF above 1 for both Dechlorane Plus isomers (11.3 for the syn- isomer and 6.5 for the anti- isomer) in a freshwater food web from South China. The TMF depends on the assignment of one species (a water snake) to the highest trophic position. Inclusion of a top predatory fish (Northern Snakehead) results in a lack of biomagnification (and even biodilution for the anti-isomer). Levels in three benthic-feeding carp species were consistently higher than potential invertebrate prey, suggesting that biomagnification may occur for some feeding relationships although this might also be influenced by sediment ingestion.

ii) Tomy *et al*. (2007) reported a lipid weight TMF value above 1 for the anti- isomer in Lake Winnipeg (TMF = 2.5, r^2 = 0.12, p = 0.04), but not for the syn- isomer, or either isomer in Lake Ontario. In Lake Winnipeg, trophic level-adjusted BMFs (based on the ratio of lipid-corrected concentrations) for four feeding relationships were all below 1 for both syn- and anti- isomers, with the exception of the Walleye/Whitefish predator-prey relationship (BMF of 11, for the anti- isomer only). In Lake Ontario, trophic level-adjusted BMFs calculated for four feeding relationships were below 1 for both isomers, with the exception of the Lake Trout/Smelt predator-prey relationship (12 for the syn- isomer and 11 for the anti- isomer).

iii) Wang *et al*. (2015) reported a statistically significant TMF of 1.9 (95 % CI: 1.1–3.4) for anti-Dechlorane Plus in an aquatic food chain in China. Concentrations in fish were much higher than in the study of Tomy *et al*. (2007), although sample sizes were small.

iv) Sun *et al.* (2015) reported a TMF for total Dechlorane Plus of 2.31 in a Chinese aquatic food web, although the regression was not statistically significant (p = 0.07). Estimated BMFs for various predator-prey combinations were in the range 1.27 to 11.8. These calculations assume that a predator consumes one prey species only, and this is unlikely to be the case in reality.

v) Several studies have attempted to investigate biomagnification potential in food chains involving birds, and some suggest BMFs above 1 for some feeding relationships or a positive trend with apparent trophic position (e.g. Yu *et al*., 2013; Barón *et al*., 2014a; Sun *et al*., 2012; Peng *et al.*, 2015). As well as the usual interpretational issues surrounding field studies, uncertainties about the relevance of lipid normalisation and trophic positioning of the sampled species mean that firm conclusions about trophic transfer in birds cannot be drawn from the available data. These studies therefore only provide equivocal evidence that contamination levels increase with trophic position in birds.

Field studies are difficult to interpret because there are many confounding factors, including variable exposure across concentration gradients in the sampled environment, small sample size and a limited choice of tissues that might not actually be representative of whole body concentrations. TMF values obtained for polluted environments close to point sources are also subject to uncertainty because organisms higher in the food chain may be receiving the substance directly via water or sediment in addition to exposure via food. Because of the confounding factors, all of these studies are assigned a low weighting in the bioaccumulation assessment.

Dechlorane Plus has been detected in human cord serum, which indicates transfer across the placenta and exposure of human foetuses. The presence of the substance in human breast milk also means that exposure can continue prior to weaning. These data have generally been obtained in areas where high exposure to the chemical occurs (e.g. e-waste recycling sites in Asia). It cannot be stated with certainty whether the presence of a substance in human tissues alone is indicative of a B or vB substance, so this information is assigned a moderate weighting for the bioaccumulation assessment.

- The REACH PBT Guidance (ECHA, 2017a) suggests a value of 0.001 mmol/kg ww [0.02 mmol/kg lipid (normalised to a lipid content of 5 %)] as a level of accumulation that is unlikely to lead to high body burdens. This is divided by a factor of 10 to account for

species differences and organ versus body differences. In the case of Dechlorane Plus (with a molecular weight of 653.73 g/mol), this "critical concentration" is equivalent to 0.65 mg/kg ww [13 mg/kg lw] (without the additional factor of 10), or 0.065 mg/kg ww [1.3 mg/kg lw] (with the additional factor of 10) (see Appendix 7).
The following maximum concentrations have been reported for environmental biota samples:

ca. 1 mg/kg ww [~95 mg/kg lw] in fish muscle (Wang *et al*., 2015);
ca. 1 mg/kg ww [~7 mg/kg lw] in terrestrial bird eggs (Zheng *et al*., 2014b);
ca. 0.5 mg/kg ww [~3.8 mg/kg lw] in terrestrial bird liver and muscle (Sun *et al*., 2012; Chen *et al*., 2013b);
ca. 3 mg/kg lw in human blood (Zhang *et al*., 2013);
1 mg/kg ww for vegetables and 0.9 mg/kg ww for grains (with a maximum for one vegetable type of 2.72 mg/kg ww) (Wang *et al*., 2013a).

In addition, a concentration of ca. 1 000 mg/kg [ca. 1 g/kg] lw in liver in Brown Rats *Rattus norvegicus* was achieved under laboratory conditions when dosed at 100 mg/kg/d via oral gavage for 90 days (Li *et al*., 2013b). Similar findings were made in Common Quail *Coturnix coturnix* (Li *et al*., 2013a), with the highest concentrations (ca. 1 750 mg/kg lw) detected in livers of the 10 mg/kg/d dose group after 90 days. None of the available laboratory fish studies using aqueous exposure are valid and they are confounded by variable exposure concentrations and potential ingestion of particulates and the substance adsorbed to food. Nevertheless, (non-steady state) concentrations up to 8.78 mg/kg ww were observed in Bluegill Sunfish *Lepomis macrochirus* (Boudreau and Rausina, 1973).

Some of these concentrations exceed the critical concentration without the factor of 10, and all exceed the critical concentration with the factor of 10. The accumulation in rat liver exceeds the highest critical concentration by a factor of around 75. On this basis, Dechlorane Plus can clearly achieve concentrations in biota that are of concern in a bioaccumulation context. This finding is assigned a high weighting in the bioaccumulation assessment.

Negative evidence

- Some field studies do not indicate biomagnification (e.g. Klosterhaus *et al*., 2012; Barón *et al*., 2013; Peng *et al*., 2014), or suggest BSAFs below 1 (Zhang *et al*., 2011c; Shen *et al*., 2011a; Wang *et al.*, 2012; He *et al*., 2014). One non-standard laboratory study also gave BSAF values below 1 for *Lumbriculus variegatus* (Li *et al.*, 2014a). However, the use of field evidence is given a low weighting in this analysis, and there are complications in the interpretation of BSAF data for highly hydrophobic substances. It may also be noted that for two studies where the BSAF was below 1, the BSAF values for Dechlorane Plus were similar to those for BDE-183 (a heptabromodiphenyl ether) in the same samples.

Significant limitations in the available regulatory data set create uncertainty in the bioaccumulation assessment. A high level of bioaccumulation can be expected based on simple screening data related to physico-chemical properties and molecular parameters. There are no definitive data from fully valid studies showing that Dechlorane Plus has a fish BCF above 5 000 L/kg, a fish BMF > 1 or BSAF > 1 in the laboratory. Evidence from field studies is conflicting; whilst some suggest TMF/BMF/BSAFs above 1, none of the studies is considered to be particularly reliable. Nevertheless, the substance is widely dispersed in both aquatic and terrestrial food chains, including top predators. In terms of the aim of protecting organisms from unpredictable adverse effects, a long depuration half-life is a key factor since substance concentrations may take a long time to decline once emissions cease. Dechlorane Plus has a long depuration half-life in fish consistent with other substances that have a fish BCF above 5 000 L/kg (supported by a long depuration half-life in mammalian liver). Levels achieved in laboratory exposures and detected in a variety of wildlife species indicate that Dechlorane Plus

can achieve a relatively high body burden in some cases, consistent with levels that may be associated with toxic effects due to baseline narcosis (see Appendix 3, 4 and 6). These are the principle reasons why the substance is concluded to meet the very bioaccumulative (vB) criteria[37] in Annex XIII of REACH.

6.2.1.3 Toxicity

6.2.1.3.1 Fulfilment of the T criterion based on human health classification

The substance does not appear to cause relevant adverse effects in mammals via oral or dietary exposure at concentrations above 1 000 mg/kg bw/d, although there are some data gaps (e.g. there are no long-term studies exceeding 90 days, which might be important given the apparently slow uptake of the substance). The dosing vehicles might also limit exposure (e.g. due to the presence of undissolved micro-crystals), such that the high "doses" might not truly reflect the degree of exposure of the organisms.

Nevertheless, based on the available data, Dechlorane Plus does not meet the classification criteria for mutagenicity, toxicity to reproduction or specific target organ toxicity. The data are considered to be conclusive but not sufficient for classification for these endpoints. Carcinogenicity data are lacking (and are not required at the registration tonnage). There is some evidence for potential liver impairment in mice (Wu *et al*., 2012), but the significance of these findings is unclear.

Based on this information, the T criterion is not fulfilled at present.

6.2.1.3.2 Fulfilment of the T criterion based on ecotoxicity data

The very limited available data do not indicate that Dechlorane Plus fulfils the T criterion for the environment. All the available aquatic toxicity studies were conducted at concentrations significantly above the solubility limit of the substance in pure water (2 ng/L), and studies using dietary and gavage exposure also used relatively high concentrations that may not be environmentally relevant. The study of Liang *et al*. (2014) suggests that the substance might induce potentially toxic effects in fish liver, but is inconclusive. However, similar findings were also observed in a study with mice via oral exposure (see Wu *et al*. (2012) in Section 4.2.1 of Appendix 1). Recent non-standard studies with adult and embryo/larval Zebrafish suggest that Dechlorane Plus has biological activity and can induce effects such as oxidative stress, thyroid hormone-related gene up-regulation and neurobehavioural changes (Hang *et al*., 2013 [ABST]; Noyes *et al*., 2015; Kang *et al*., 2016; Chen *et al*., 2017). However, the reliability of the findings is uncertain, their links to population-relevant adverse apical effects is unknown, and benchmarking with other substances would be beneficial (where this has been done, the level of response seems lower for Dechlorane Plus than (chlorinated) phosphate esters). In general, the

[37] A substance fulfils the vB criterion when the BCF in aquatic species is higher than 5 000 L/kg; or other information on the bioaccumulation potential [is available] … such as:

- Results from a bioaccumulation study in terrestrial species;
- Data from scientific analysis of human body fluids or tissues, such as blood, milk, or fat;
- Detection of elevated levels in biota, in particular in endangered species or in vulnerable populations, compared to levels in their surrounding environment;
- Results from a chronic toxicity study on animals;
- Assessment of the toxicokinetic behaviour of the substance;
- Information on the ability of the substance to biomagnify in the food chain, where possible expressed by biomagnification factors or trophic magnification factors.

NOEC from these studies appears to be above 0.01 mg/L (for aqueous exposures), i.e. above the T criterion (and also significantly above the solubility limit in pure water).

It is highly unlikely that effects would be observed in short- or long-term toxicity tests involving aqueous exposure, as there would be difficulties in maintaining test concentrations given the very low water solubility and high hydrophobicity[38]. A more appropriate test might be a long-term fish toxicity study with relevant life stages using dietary exposure, although a standard test guideline does not exist.

Several studies show that maternal uptake and transfer to eggs can occur in birds (e.g. Guerra *et al*., 2011; Munoz-Arnanz *et al*., 2011; Gauthier *et al.*, 2007; Barón *et al*., 2014a; and Zheng *et al*., 2014b) and fish (e.g. Wu *et al*., 2013; Sühring *et al*., 2015). The studies of Zhang *et al*. (2011a) and Zeng *et al*. (2014a) also show that the substance can cross the blood-brain barrier, is present in gonads and can be passed from females to eggs in fish. Li *et al*. (2014b) found that the substance can cross the blood-brain barrier in frogs. Similarly, detection of these substances in human cord serum indicates transfer across the placenta and exposure of foetuses, and presence in human breast milk also means that exposure can continue prior to weaning (e.g. Ben *et al*. (2014) and Zhou *et al*. (2014)). Sensitive life stages and tissues are therefore exposed to the substance following prolonged exposure, and there is a possibility that they might experience subtle but important adverse effects that are not detectable in the studies currently available (e.g. because of their short duration).

No sediment toxicity studies are available.

Suitable long-term soil organism toxicity data are also unavailable, although two studies investigating molecular and biomarker end points have detected signs of oxidative stress and other damage (including on DNA) in the earthworm *Eisenia fetida* following exposures up to 14 days (Zhang *et al*., 2014) and 28 days (Yang *et al*., 2014), with a 28-d NOEC below 0.1 mg/kg dw. Although these findings cannot be related directly to adverse population-relevant apical effects, they imply that effects (e.g. on behaviour and reproduction) cannot be excluded in earthworms over the longer term.

Results from long-term or reproductive avian toxicity studies may also be used assess the T criterion. No mortalities occurred in male Common Quail (*Coturnix coturnix*) exposed up to 1 000 mg/kg bw/d for 90 days (Li *et al.*, 2013a). No effects were observed in Domestic Chicken (*Gallus gallus domesticus*) embryonic hepatocytes *in vitro* or embryos following egg injection (although there was a possible dose-dependent decrease in pipping success, the results were within the historical control range so cannot be considered significant). The available data do not suggest significant toxicity in birds, but a standard test guideline study is not available so the findings cannot be considered conclusive.

Dechlorane Plus is structurally related to known pesticides such as heptachlor (CAS no. 76-44-8) and chlordane (CAS no. 57-74-9) (see Section 1.4). However, the structures and physico-chemical properties of the analogues and Dechlorane Plus are not similar enough to conclude that Dechlorane Plus would have similar toxic properties.

In summary, the available information is insufficient to make a full assessment of the T criterion based on ecotoxicity data.

[38] Indications of effects on growth of Sea Lettuce (*Ulva pertusa*) have been reported in a 14-d bioconcentration study by Zhao *et al*. (2014). However, the results of this study are not considered to be sufficiently robust to conclude that the T criterion is met.

6.2.1.4 Additional considerations

As summarised in Appendix 2, several studies have detected 1,3- or 1,5-Dechlorane Plus monoadduct (DPMA) in environmental samples such as sediment and fish (e.g. Sverko *et al*., 2010b; Guerra *et al*., 2011; Tomy *et al*., 2013; Sühring *et al*., 2014; Wang *et al*., 2015; Wolschke *et al*., 2015; Rjabova *et al*., 2016). In some cases, the concentrations of DPMA isomers are greater than the total Dechlorane Plus concentration in the same samples. In addition, it is possible that failure to use a non-destructive clean-up procedure for sample preparation could lead to under-reporting of this substance (Rjabova *et al*., 2016).

Whilst there is uncertainty in the QSAR predictions, 1,3-/1,5-DPMA screens as being potentially PBT and/or vPvB. No information is available on mammalian toxicity, but if it reacts like aldrin or heptachlor via epoxidation in the environment, it could be neurotoxic and/or cause hepatotoxicity.

Given its structure, Dechlorane Plus is the only likely source of DPMA in the environment. There is no information on reaction rates or amounts that can be formed under relevant environmental conditions. Some other possible transformation products (e.g. hexachloronorbornadiene) also screen as potentially PBT and/or vPvB.

A definitive conclusion would require experimental data to confirm that these degradants are formed in relevant amounts in standard transformation studies, and also to confirm their properties. However, DPMA in particular flags an additional relevant concern as it is has been detected in biota (including in the Antarctic).

6.2.2 Summary and overall conclusions on the PBT and vPvB properties

Persistence

Based on the weight of evidence of the data available, it is concluded that Dechlorane Plus meets the criteria for vP in Annex XIII of REACH. This is based on:

- modelling of degradation potential and microbial metabolic pathways which suggests that biodegradation is likely to be very slow; and
- a low probability that it will degrade any faster than structural analogues that are considered to be very persistent under the Stockholm Convention.

This conclusion is also supported by the very low water solubility (suggesting limited bioavailability to micro-organisms once bound to solid matrices), monitoring data indicating that Dechlorane Plus can persist in sediments (a major sink) for many years, lack of evidence of biotransformation in fish (supporting the premise that the molecule is metabolically recalcitrant) and widespread occurrence in remote regions.

Bioaccumulation

Using a weight of evidence assessment of the data available, Dechlorane Plus meets the vB criteria in Annex XIII of REACH. This is based on:

- the long-depuration half-life determined in fish feeding studies which is indicative of a BCF above 5 000 L/kg, by comparison with other substances (supported by a long depuration half-life in mammalian liver);
- numerous studies that show that the substance is widely dispersed in freshwater, marine and terrestrial food chains, including top predators; and
- evidence that the substance can exceed levels in biota that are of concern based on

critical body burden considerations related to baseline narcosis.

This conclusion is supported by the detection of Dechlorane Plus in human blood, placenta and breast milk.

Toxicity

Based on the available ecotoxicity and mammalian data, Dechlorane Plus does not currently meet the T criterion. Long-term toxicity studies using relevant life stages of fish (via diet), sediment or soil organisms, and/or birds could be performed to clarify whether adverse effects can occur via these exposure routes. However, as the substance meets both the vP and vB criteria, these are not scientifically necessary for environmental risk management purposes.

Other concerns

The substances 1,3- and 1,5-Dechlorane Plus monoadduct (DPMA) have been detected in the environment, sometimes at higher concentrations than Dechlorane Plus in the same samples. DPMA might be under-reported because destructive sample preparation methods may degrade it. Dechlorane Plus is the only likely source of these two substances, although there is no information on reaction rates or amounts that can be formed under relevant environmental conditions. Based on predictive models, DPMA screens as being potentially PBT and vPvB on the basis of QSAR (although some of the predictions are uncertain). No information is available on its mammalian toxicity, but due to structural similarities to aldrin or heptachlor it might be epoxidised in the environment to form a substance that could be neurotoxic and/or cause hepatotoxicity. Experimental data would be needed to confirm these properties. However, as a degradation product of Dechlorane Plus, any concerns about DPMA would be alleviated by the identification of Dechlorane Plus as a substance of very high concern.

In conclusion, despite the lack of definitive data, Dechlorane Plus is identified as a vPvB substance according to Art. 57(e) of REACH by comparing all relevant and available information listed in Annex XIII of REACH with the criteria set out in the same Annex, in a weight-of-evidence determination.

REFERENCES

References for Main Report and Appendices

Arinaitwe K, Muir DCG, Kiremire BT, Fellin P, Li H and Teixeira C (2014). Polybrominated diphenyl ethers and alternative flame retardants in air and precipitation samples from the Northern Lake Victoria Region, East Africa. Environmental Science and Technology, 48 (3), 1458-1466.

Arnot JA and Gobas FAPC (2003). A generic QSAR for assessing the bioaccumulation potential of organic chemicals in aquatic food webs. QSAR and Combinatorial Science, 22, 337-345.

Arnot JA, Mackay D and Bonnell M (2008). Estimating metabolic biotransformation rates in fish from laboratory data. Environ. Toxicol. Chem., 27, 341–351.

Arnot JA, Meylan W, Tunkel J, Howard PH, Mackay D, Bonnell M and Boethling RS (2009). A quantitative structure-activity relationship for predicting metabolic biotransformation rates for organic chemicals in fish. Environ. Toxicol. Chem., 28, 1168–1177.

Arnot JA and Quinn CL (2015). Development and evaluation of a database of dietary bioaccumulation test data for organic chemicals in fish. Environ. Sci. Technol., 49 (8), 4783–4796.

Barón E, Eljarrat E and Barceló D (2012). Analytical method for the determination of halogenated norbornene flame retardants in environmental and biota matrices by gas chromatography coupled to tandem mass spectrometry. J Chromatogr A., 1248, 154-60.

Barón E, Rudolph I, Chiang G, Barra R, Eljarrat E and Barceló D (2013). Occurrence and behavior of natural and anthropogenic (emerging and historical) halogenated compounds in marine biota from the Coast of Concepcion (Chile). Science of the Total Environment, 461-462, 258-264.

Barón E, Máñez M, Andreu A, Sergio F, Hiraldo F, Eljarrat E and Barceló D (2014a). Bioaccumulation and biomagnification of emerging and classical flame retardants in bird eggs of 14 species from Doñana Natural Space and surrounding areas (south-western Spain). Environ. Int., 68, 118-126.

Barón E, Santín G, Eljarrat E and Barceló D (2014b). Occurrence of classic and emerging halogenated flame retardants in sediment and sludge from Ebro and Llobregat river basins (Spain). Journal of Hazardous Materials, 265, 288-295.

Barón E , Giménez J, Verborgh P, Gauffier P, De Stephanis R, Eljarrat E and Barceló D (2015a). Bioaccumulation and biomagnification of classical flame retardants, related halogenated natural compounds and alternative flame retardants in three delphinids from Southern European waters. Environmental Pollution, 203, 107-115.

Barón E, Hauler C, Gallistl C, Giménez J, Gauffier P, Castillo JJ, Fernández-Maldonado C, De Stephanis R, Vetter W, Eljarrat E and Barceló D (2015b). Halogenated natural products in dolphins: brain–blubber distribution and comparison with halogenated flame retardants. Environ. Sci. Technol. In press. DOI: 10.1021/acs.est.5b02736.

Ben Y-J, Li X-H, Yang Y-L, Li L, Di J-P, Wang W-Y, Zhou R-F, Xiao K, Zheng M-Y and Tian Y (2013). Dechlorane plus and its dechlorinated analogs from an e-waste recycling center in maternal serum and breast milk of women in Wenling, China. Environ. Pollut., 173, 176−181.

Ben Y-J, Li X-H, Yang Y-L, Li L, Zheng M-Y, Wang W-Y and Xu X-B (2014). Placental transfer of Dechlorane Plus in mother−infant pairs in an e–waste recycling area (Wenling, China). Environ. Sci. Technol., 48, 5187−5193.

Boudreau J (1973). Report to Hooker Chemical Company: Determination of the biodegradability of Dechloranes Plus, 602 and 604. Industrial Bio-Test Laboratories Inc., IBT No. 631-02353, 5 July 1973. Testing laboratory: Industrial Bio-Test Laboratories Inc., Northbrook, Illinois, USA. Report no.: IBT No. 631-02353. Owner company: Hooker Chemical Corporation. Report date: 1973-07-05.

Boudreau J and Rausina G (1973). Report to Hooker Chemical Corporation, Industrial Chemicals Division: Thirty day static biological magnification studies with Dechloranes 602, 604, and Plus 515 in bluegill sunfish. Industrial Bio-Test Laboratories Inc., IBT Nos. 665-03736 and 631-02353, 25 October 1973. Testing laboratory: Industrial Bio-Test Laboratories Inc.,

Northbrook, Illinois, USA. Report no.: IBT Nos. 665-03736 and 631-02353. Owner company: Hooker Chemical Corporation. Report date: 1973-10-25.

Brasseur C, Pirard C, Scholl G, De Pauw E, Viel J-F, Shen L, Reiner EJ and Focant J-F (2014). Levels of dechloranes and polybrominated diphenyl ethers (PBDEs) in human serum from France. Environment International, 65, 33-40.

Brock WJ, Schroeder RE, McKnight CA, Van Steenhouse JL and Nyberg JM (2010). Oral repeat dose and reproductive toxicity of the chlorinated flame retardant Dechlorane Plus. Int. J. Toxicol., 29, 582–593.

Brooke D N, Crookes M J and Merckel D A S (2012). Methods for predicting the rate constant for uptake of organic chemicals from water by fish. Environ. Toxicol. Chem., 31, 2465-2471.

Brown T and Wania F (2008). Screening chemicals for the potential to be persistent organic pollutants: a case study of Arctic contaminants. Environ. Sci. Technol., 42, 5202–5209.

Budavari S (2001). *The Merck Index: An encyclopaedia of chemicals, drugs and biological,* 13th Edition, Merck.

Burkhard LP, Cook PM and Lukasewycz MT (2004). Biota-sediment accumulation factors for polychlorinated biphenyls, dibenzo-p-dioxins, and dibenzofurans in southern Lake Michigan lake trout (*Salvelinus namaycush*). Environ. Sci. Technol., 38, 5297–5305.

Byer JD, Lebeuf M, Alaee M, Brown RS, Trottier S, Backus S, Keir M, Casselman J and Hodson PV (2013). Spatial trends of organochlorinated pesticides, polychlorinated biphenyls, and polybrominated diphenyl ethers in Atlantic anguillid eels. Chemosphere, 90, 1719–1728.

Canada. 2016a. Dept. of the Environment. Dept. of Health. Draft Screening Assessment Certain Organic Flame Retardants Grouping 1,4:7,10-Dimethanodibenzo[a,e]cyclooctene, 1,2,3,4,7,8,9,10,13,13,14,14-dodecachloro-1,4,4a,5,6,6a,7,10,10a,11,12,12a-dodecahydro-Dechlorane Plus (DP), Chemical Abstracts Service Registry Number 13560-89-9.

CECBP (2008). Brominated and chlorinated organic chemical compounds used as flame retardants. Materials for the December 4-5, 2008 Meeting of the California Environmental Contaminant Biomonitoring Program (CECBP) Scientific Guidance Panel (SGP). Available online at http://oehha.ca.gov/multimedia/biomon/pdf/120408flamedoc.pdf.

CEH (2014). Email from R Shore (CEH) to S Dungey (Environment Agency) of 11 July 2014.

CEMC (2004). The Canadian Centre for Environmental Modelling and Chemistry Level III Model. Version 2.80 - May 2004. http://www.trentu.ca/academic/aminss/envmodel/models/L3280.html.

Cequier E, Marcé RM, Becher G and Thomsen C (2013). Determination of emerging halogenated flame retardants and polybrominated diphenyl ethers in serum by gas chromatography mass spectrometry. J. Chromatogr. A., 1310, 126-132.

Cequier E, Marcé RM, Becher G and Thomsen C (2015). Comparing human exposure to emerging and legacy flame retardants from the indoor environment and diet with concentrations measured in serum. Environment International, 74, 54-59.

Chabot-Giguère B, Letcher RJ and Verreault J (2013). *In vitro* biotransformation of decabromodiphenyl ether (BDE-209) and Dechlorane Plus flame retardants: A case study of ring-billed gull breeding in a pollution hotspot in the St. Lawrence River, Canada. Environment International, 55, 101–108.

Chen D, Letcher RJ and Martin P (2012a). Flame retardants in eggs of American kestrels and European starlings from southern Lake Ontario region (North America). Journal of Environmental Monitoring, 14, 2870-2876.

Chen D, Letcher RJ, Burgess NM, Champoux L, Elliott JE, Hebert CE, Martin P, Wayland M, Weseloh DVC, Wilson L (2012b). Flame retardants in eggs of four gull species (*Laridae*) from breeding sites spanning Atlantic to Pacific Canada. Environmental Pollution, 168, 1-9.

Chen D, Martin P, Burgess NM, Champoux L, Elliott JE, Forsyth DJ, Idrissi A and Letcher RJ (2013a). European Starlings (*Sturnus vulgaris*) suggest that landfills are an important source of bioaccumulative flame retardants to Canadian terrestrial ecosystems. Environ. Sci. Technol., 47, 12238−12247.

Chen D, Wang Y, Yu L, Luo X, Mai B and Shaoshan Li (2013b). Dechlorane Plus flame retardant in terrestrial raptors from northern China. Environmental Pollution, 176, 80-86.

Chen C-L, Tsai D-Y and Ding W-H (2014). Optimisation of matrix solid-phase dispersion for the

determination of Dechlorane compounds in marketed fish. Food Chemistry, 164, 286-292.
Chen K, Zheng J, Yan X, Yu L, Luo X, Peng X, Yu Y, Yang Z and Mai B (2015). Dechlorane Plus in paired hair and serum samples from e-waste workers: Correlation and differences. Chemosphere, 123, 43-47.
Chen X, Dong Q, Chen Y, Zhang Z, Huang C, Zhu Y and Zhang Y (2017). Effects of Dechlorane Plus exposure on axonal growth, musculature and motor behavior in embryo-larval zebrafish. Environmental Pollution, 224, 7-15.
Chou TW, Liu DHW, Mabey WR, MItoma C and Smith JH (1979). Metabolism and environmental screening studies on Dechlorane Plus. SRI International, SRI Project LSC-8060, 25 April 1979. Testing laboratory: SRI International, Menlo Park, California, USA. Report no.: LSC-8060. Owner company: Hooker Chemical Corporation, Hooker Research Center. Report date: 1979-04-25.
Crump D, Chiu S, Gauthier LT, Hickey NJ, Letcher RJ and Kennedy SW (2011). The effects of Dechlorane Plus on toxicity and mRNA expression in chicken embryos: A comparison of *in vitro* and *in ovo* approaches. Comparative Biochemistry and Physiology, Part C, 154, 129–134.
Dearth MA and Hites RA (1991). Complete analysis of technical chlordane using negative ionization mass spectrometry. Environ. Sci. Technol., 25, 245–254.
de la Torre A, Pacepavicius G, Shen L, Reiner E, Jimenez B, Alaee M and Martinez MA (2010). Dechlorane Plus and related compounds in Spanish air. Organohalogen Compd., 72, 929–932.
de la Torre A, Sverko E, Alaee M and Martínez MA (2011). Concentrations and sources of Dechlorane Plus in sewage sludge. Chemosphere, 82, 692–697.
de la Torre A, Alonso MB, Martínez MA, Sanz P, Shen L, Reiner EJ, Lailson-Brito J, Torres JPM, Bertozzi C, Marigo J, Barbosa L, Cremer M, Secchi E, Malm O, Eljarrat E and Barceló D (2012). Dechlorane-related compounds in Franciscana dolphin (*Pontoporia blainvillei*) from southeastern and southern coast of Brazil. Environ. Sci. Technol., 46, 12364−12372.
Dimitrov S, Dimitrova N, Parkerton T, Comber M, Bonnell M and Mekenyan O (2005). Base-line model for identifying the bioaccumulation potential of chemicals. SAR QSAR Environ. Res., 16(6), 531–554.
EA [Environment Agency] (2009a). Environmental Risk Assessment Report: Octamethylcyclotetrasiloxane. Environment Agency Science Report, SCHO0309BPQZ-E-P, April 2009. Environment Agency, Bristol, UK. Available at: https://www.gov.uk/government/uploads/system/uploads/attachment_data/file/290565/scho0309bpqz-e-e.pdf.
EA (2009b). Environmental Risk Assessment Report: Decamethylcyclopentasiloxane. Environment Agency Science Report, SCHO0309BPQX-E-P, April 2009. Environment Agency, Bristol, UK. Available at: https://www.gov.uk/government/uploads/system/uploads/attachment_data/file/290561/scho0309bpqx-e-e.pdf.
EA (2009c). Calculation of Molecular Dimensions Related to Indicators for Low Bioaccumulation Potential. Science report SCHO0109BPGT-E-P, January 2009. Environment Agency, Bristol, UK. Available at: https://www.gov.uk/government/uploads/system/uploads/attachment_data/file/291060/scho0109bpgt-e-e.pdf.
EA (2011). Estimation of fish bioconcentration factor (BCF) from depuration data. Environment Agency, Bristol, UK. Available at: https://www.gov.uk/government/uploads/system/uploads/attachment_data/file/291527/scho0811buce-e-e.pdf.
EA (2012). Depuration Rate Constant as an Indicator of Bioaccumulation Potential. Environment Agency, Bristol, UK. Available from https://www.gov.uk/government/uploads/system/uploads/attachment_data/file/291525/LIT_7371_44228a.pdf.
EA (2013). Review of the BCFBAF model within EPIWEBv4. Final report February 2013 (unpublished). Environment Agency, Bristol, UK.

EA (2014). PBT report for D5. Paper for agreement at the Member State Committee. October 2014. Available at: http://echa.europa.eu/documents/10162/9c211198-2f6d-40d5-8569-7b87a4c3782d.

EC [European Communities] (undated). European Union Risk Assessment Report: Hexachlorocyclopentadiene. Final Approved Version, July 2007, European Commission. Available at: https://echa.europa.eu/documents/10162/8a93cd69-5a82-4c2f-b0bd-0bbe059074a2

EC (2001a). European Union Risk Assessment Report: Diphenyl ether, pentabromo derivative. European Chemicals Bureau, Institute of Health and Consumer Protection, European Commission. Available at: http://echa.europa.eu/documents/10162/781ee1e9-6c90-467e-998b-8910ca2793e5.

EC (2001b). European Union Risk Assessment Report: Cumene. European Chemicals Bureau, Institute of Health and Consumer Protection, European Commission. Available at: http://echa.europa.eu/documents/10162/c44474a0-e926-451d-9efd-810b230008f4

EC (2004). European Union Risk Assessment Report: Cyclohexane. European Chemicals Bureau, Institute for Health and Consumer Protection, European Commission. Available at: https://echa.europa.eu/documents/10162/d99cee39-82e6-4754-aac1-497106c9bd7c.

EC (2007). European Union Risk Assessment Report: Ethylbenzene. European Chemicals Bureau, Institute of Health and Consumer Protection, European Commission. Available at: http://echa.europa.eu/documents/10162/f9b4577b-c57f-439b-b15a-9e268b1d0a58.

EC (2008). European Union Risk Assessment Report: Anthracene. European Chemicals Bureau, Institute of Health and Consumer Protection, European Commission. Available at: http://echa.europa.eu/documents/10162/08a49c89-9171-4cb2-8366-108601ac565c.

ECHA [European Chemicals Agency] (2008a). Member State Committee Support Document for Identification of Hexabromocyclododecane and all Major Diastereoisomers Identified as a Substance of Very High Concern. Adopted on 8 October 2008. Available at: http://echa.europa.eu/documents/10162/d12ef98c-3fb9-484b-b354-4a2c74931cdd.

ECHA (2008b). Member State Committee Support Document for Identification of alkanes, C_{10-13}, chloro as a Substance of Very High Concern. Adopted on 8 October 2008. Available at: http://echa.europa.eu/documents/10162/414fa327-56a1-4b0c-bb0f-a6c40e74ece2.

ECHA (2008c). Member State Committee Support Document for Identification of 5-tert-butyl-2,4,6-trinitro-m-xylene as a Substance of Very High Concern. Adopted on 8 October 2008. Available at: http://echa.europa.eu/documents/10162/9ac9a275-d35c-4d24-b4e7-9630642b1d54.

ECHA (2012a). Support Document for Identification of Bis(pentabromophenyl) Ether as a Substance of Very High Concern Because of its PBT/vPvB Properties. Adopted on 29 November 2012. Available at: http://echa.europa.eu/web/guest/identification-of-svhc?search_criteria=decabromodiphenyl+ether.

ECHA (2012b). Member State Committee Support Document for Identification of Henicosafluoroundecanoic Acid as a Substance of Very High Concern Because of its vPvB Properties. Adopted on 13 December 2012. Available at: http://echa.europa.eu/documents/10162/e359141e-e5cf-4ddf-b197-7701ea563b0f.

ECHA (2012c). Member State Committee Support Document for Identification of Tricosafluorododecanoic Acid as a Substance of Very High Concern Because of its vPvB Properties. Adopted on 13 December 2012. Available at: http://echa.europa.eu/documents/10162/4047c3cf-cff2-45e1-a56d-563bf4088ad5.

ECHA (2012d). Member State Committee Support Document for Identification of Heptacosafluorotetradecanoic Acid as a Substance of Very High Concern Because of its vPvB Properties. Adopted on 13 December 2012. Available at: http://echa.europa.eu/documents/10162/32710c65-b872-4630-a59e-4f31e2d0a9cb.

ECHA (2014a). Member State Committee Support Document for Identification of 2-(2H-Benzotriazol-2-yl)-4,6-ditertpentylphenol (UV-328) as a Substance of Very High Concern Because of its PBT/vPvB Properties. Adopted on 27 November 2014. Available at:

http://echa.europa.eu/documents/10162/d5b54a40-4ddf-4892-a25a-442dfab8f9f3.

ECHA (2014b). Member State Committee Support Document for Identification of 2-Benzotriazol-2-yl-4,6-di-tert-butylphenol (UV-320) as a Substance of Very High Concern Because of its PBT/vPvB Properties. Adopted on 27 November 2014. Available at: http://echa.europa.eu/documents/10162/8b110e69-c9aa-4601-b431-ee43177e0ee8.

ECHA (2017a). Guidance on Information Requirements and Chemical Safety Assessment. Chapter R.11: PBT/vPvB Assessment. Version 3.0. June 2017. European Chemicals Agency.

ECHA (2017b). Guidance on Information Requirements and Chemical Safety Assessment. Chapter R.7b: Endpoint specific guidance. Version 4.0. June 2017. European Chemicals Agency.

ECHA (2017c). Guidance on Information Requirements and Chemical Safety Assessment. Chapter R.7c: Endpoint specific guidance. Version 3.0. June 2017. European Chemicals Agency.

ECHA (2017d). Substance Evaluation Conclusion document for Methylcyclohexane, April 2017. European Chemicals Agency. Available at: https://echa.europa.eu/documents/10162/8746a7eb-aa55-e66d-e1f1-4ee4393f0bc9.

EFSA [European Food Standards Agency] (unpubl. a) Draft Assessment Report for technical endosulfan dated December 1999. Unpublished.

EFSA (unpubl. b) Draft Assessment Report (addendum) for technical endosulfan dated December 2003. Unpublished.

EFSA (2007). Opinion of the Scientific Panel on Contaminants in the Food Chain on a Request from the European Commission Related to Heptachlor as an Undesirable Substance in Animal Feed. Question N° EFSA-Q-2005-184. The EFSA Journal, 478, 1-48.

Eljarrat E (2014). Classical versus Emerging Flame Retardants. Presentation given to 8th Network Conference on Persistent Organic Pollutants, Birmingham, UK on 8 May 2014. Available at: http://www.birmingham.ac.uk/Documents/college-les/gees/conferences/nercpops/Conference2014/Classical%20vs%20emerging%20flame%20retardants.pdf. Accessed 15 July 2014.

EUSES 2.1.2 (2012). EUSES, the European Union System for the Evaluation of Substances, version 2.1.2. National Institute of Public Health and the Environment (RIVM), the Netherlands. Available via the European Chemicals Bureau http://ecb.jrc.it.

Fang M, Kim J-C and Chang Y-S (2014). Investigating Dechlorane Plus (DP) distribution and isomer specific adsorption behavior in size fractionated marine sediments. Science of the Total Environment, 481, 114–120.

Gara MJ and Rawisina G (1975). Report to Hooker Chemical Corporation, Industrial Chemicals Division: Thirty day dynamic bioaccumulation studies with Dechlorane Plus 25 in bluegill sunfish. Industrial Bio-Test Laboratories Inc., IBT nos. 621-06768 and 631-04618, 08 December 1975. Testing laboratory: Industrial Bio-Test Laboratories Inc., Northbrook, Illinois, USA. Report no.: IBT nos. 621-06768 and 631-04618. Owner company: Hooker Chemicals Corporation. Report date: 1975-12-07.

Gauthier LT, Hebert CE, Weseloh DVC and Letcher RJ (2007). Current-use flame retardants in the eggs of herring gulls (Laurus argentatus) from the Laurentian Great Lakes. Environ Sci Technol, 41, 4561-4567.

Gauthier LT and Letcher RJ (2009). Isomers of Dechlorane Plus flame retardant in the eggs of herring gulls (*Larus argentatus*) from the Laurentian Great Lakes of North America: Temporal changes and spatial distribution. Chemosphere, 75, 115–120.

Gentes M-L, Letcher RJ, Caron-Beaudoin É and Verreault J (2012). Novel flame retardants in urban-feeding ring-billed gulls from the St. Lawrence River, Canada. Environ. Sci. Technol., 46, 9735−9744.

Gobas FAPC, Kelly BC and Arnot JA (2003). Quantitative structure activity relationships for predicting the bioaccumulation of POPs in terrestrial food-webs. QSAR Comb. Sci., 22, 329–336.

Gobas FAPC, Xu S, Kozerski G, Powell DE, Woodburn KB and Mackay D (2015). Fugacity and acitivity analysis of the bioaccumulation and environmental risks of decamethylpentasiloxane (D5). Environmental Toxicology and Chemistry, 34, 2723-2731.

Gong N, Shao K, Hang X, Zhao L, Zhong R, Zhao E, Li G and Sun Y (2013). Effect of Dechlorane Plus on *Pseudokirchneriella subcapitata* by flow cytometry. Organohalogen Compounds, 75, 200-204. http://www.dioxin20xx.org/pdfs/2013/86.pdf

Goss KU, Brown TN and Endo S (2013). Elimination half-life as a metric for the bioaccumulation potential of chemicals in aquatic and terrestrial food chains. Environ. Toxicol. Chem., 32, 1663-1671.

Government of Canada (2016). Draft Screening Assessment Certain Organic Flame Retardants Substance Grouping: 1,4:7,10-Dimethanodibenzo[a,e]cyclooctene, 1,2,3,4,7,8,9,10,13,13,14,14-dodecachloro-1,4,4a,5,6,6a,7,10,10a,11,12,12a-dodecahydro- [Dechlorane Plus (DP)]. Environment and Climate Change Canada & Health Canada. October 2016. http://www.ec.gc.ca/ese-ees/default.asp?lang=En&n=FDE6572D-1.

Guerra P, Fernie K, Jiménez B, Pacepavicius G, Shen L, Reiner EJ, Eljarrat E, Barcelo D and Alaee M (2011). Dechlorane Plus and related compounds in peregrine falcon (*Falco peregrinus*) eggs from Canada and Spain. Environ. Sci. Technol., 45, 1284–1290.

Hang XM, Jiang Y, Liu Y, Jia HL and Sun YQ (2013). An integrated study on the toxicity of Dechlorane Plus in zebrafish. Organohalogen Compounds, 75, 1085-1089. http://www.dioxin20xx.org/pdfs/2013/5154.pdf

Hawker DW and Connell DW (1986). Bioconcentration of lipophilic compounds by some aquatic organisms. Ecotoxicology and Environmental Safety, 11, 184-197.

He S, Li M, Jin J, Wang Y, Bu Y, Xu M, Yang X and A Liu (2013). Concentrations and trends of halogenated flame retardants in the pooled serum of residents of Laizhou Bay, China. Environmental Toxicology and Chemistry, 32, 1242–1247.

He M-J, Luo X-J, Wu J-P, Chen S-J, Wei S-Q and Mai B-X (2014). Isomers of Dechlorane Plus in an aquatic environment in a highly industrialized area in Southern China: Spatial and vertical distribution, phase partition, and bioaccumulation. Science of the Total Environment, 481, 1–6.

Hoh E, Zhu L and Hites RA (2006). Dechlorane Plus, a chlorinated flame retardant, in the Great Lakes. Environ. Sci. Technol., 40, 1184-1189.

Hooker Chemical Corporation (1975). Report to Hooker Chemical Corporation: 28-day subacute dust inhalation toxicity study with Dechlorane Plus 25 in rats. Industrial Bio-Test Laboratories Inc., IBT No. 663-06279, 18 June 1975. Testing laboratory: Industrial Bio-Test Laboratories Inc., Northbrook, Illinois, USA. Report no.: IBT No. 663-06279. Owner company: Hooker Chemical Corporation. Report date: 1975-06-13.

Houde M, Berryman D, de Lafontaine Y and Verreault J (2014). Novel brominated flame retardants and dechloranes in three fish species from the St. Lawrence River, Canada. Sci Total Environ., 479-480, 48-56.

Huber S, Warner NA, Nygård T, Remberger M, Harju M, Uggerud HT, Kaj L and Hanssen L (2015a). A broad cocktail of environmental pollutants found in eggs of three seabird species from remote colonies in Norway, Environmental Toxicology and Chemistry, 34, 1296-308.

Huber S, Nygård T, Warner NA, Remberger M, Harju M, Uggerud HT, Kaj L, Schlabach M and Hanssen L (2015b). Kartlegging av miljøgifter i sjøfuglegg fra Sklinna og Røst. Norwegian Institute for Air Research. NILU OR 8/2014. Available at: http://www.miljodirektoratet.no/Documents/publikasjoner/M7/M7.pdf.

Inoue Y, Hashizume N, Yoshida T, Murakami H, Suzuki Y, Koga Y, Takeshige R, Kikushima R, Yakata N and Masanori O (2012). Comparison of bioconcentration and biomagnification factors for poorly water-soluble chemicals using common carp (*Cyprinus carpio*) Archives of Environmental Contamination and Toxicology, 63, 241-248.

Ismail N, Gewurtz SB, Pleskach K, Whittle M, Helm PA, Marvin CH and Tomy GT (2009). Brominated and chlorinated flame retardants in Lake Ontario, Canada, Lake Trout (*Salvelinus namaycush*) between 1979 and 2004 and possible influences of food-web changes. Environ. Toxicol. Chem., 28, 910–920.

Jia H, Sun Y, Liu X, Yang M, Wang D, Qi H, Shen L, Sverko E, Reiner EJ and Li Y-F (2011). Concentration and bioaccumulation of Dechlorane compounds in coastal environment of northern China. Environ. Sci. Technol., 45, 2613–2618.

Jotz MM and Mitchell AD (1980). An evaluation of mutagenic potential of Hooker Research Center compound Dechlorane Plus employing the L5178Y TK+/- mouse lymphoma assay. SRI

International, SRI Project LSC-8971, 22 February 1980. Testing laboratory: SRI International, Menlo Park, California, USA. Report no.: LSC-8971. Owner company: Hooker Chemical Corporation, Hooker Research Center. Report date: 1980-02-21.

Kaj L, Norstrom K, Egelrud L, Remberger M, Lilja K and Brorstrom-Lunden E (2010). Results from the Swedish National Screening Programme 2009, subreport 2: Dechlorane Plus. IVL Swedish Environmental Research Institute Ltd, Stockholm. November 2010. http://www.ivl.se/webdav/files/B-rapporter/B1950.pdf.

Kakimoto K, Nagayoshi H, Yoshida J, Akutsu K, Konishi Y, Toriba A and Hayakawa K (2012). Detection of Dechlorane Plus and brominated flame retardants in marketed fish in Japan. Chemosphere, 89, 416–419.

Kang H, Moon H-B and Choi K (2016). Toxicological responses following short-term exposure through gavage feeding or water-borne exposure to Dechlorane Plus in zebrafish (*Danio rerio*). Chemosphere, 146, 226-232.

Kang JH, Kim JC and Chang YS (2009). Dechlorane Plus in fish from urban-industrial rivers. Organohalogen Compd., 71, 1356–1359.

Kang JH, Kim JC, Jin GZ, Park H, Baek SY and Chang YS (2010). Detection of Dechlorane Plus in fish from urban industrial rivers. Chemosphere, 79, 850-854.

Kang J-H, Kim J-T, Hur SD, Kim J-H and Chang Y-S (2013). Dechlorane Plus in Antarctic biota in King George Island, Antarctica. Organohalogen Compounds, 75, 1066-1069.

Kelly BC, Ikonomou MG, Blair JD, Morin AE and Gobas FAPC (2007). Food web–specific biomagnification of persistent organic pollutants. Science, 317, 236-239.

Kim JT, Son MH, Kang JH, Kim JH, Jung JW and Chang YS (2015). Occurrence of Legacy and New Persistent Organic Pollutants in Avian Tissues from King George Island, Antarctica. Environmental Science and Technology, 2015, 49, 13628–13638.

Klasmeier J, Matthies M, MacLeod M, Fenner K, Scheringer M, Stroebe M, Le Gall AC, McKone TE, van de Meent D and Wania F (2006). Application of multimedia models for screening assessment of long-range transport potential and overall persistence. Environmental Science & Technology, 40, 53–60.

Klosterhaus S L, Stapleton H M, La Guardia M J and Greig D J (2012). Brominated and chlorinated flame retardants in San Francisco Bay sediments and wildlife. Environment International, 47, 56–65.

Kolic TM, Shen L, MacPherson K, Fayez L, Gobran T, Helm PA, Marvin CH, Arsenault G and Reiner EJ (2009). The analysis of halogenated flame retardants by GC–HRMS in environmental samples. Journal of Chromatographic Science, 47, 83-91.

Körner W, Huber D, Wanner A, Hübner G, Gierig M, Ripperger D and Fischer K (2011). Novel brominated flame retardants and dechlorane plus in sewage sludge in the state of Bavaria, Germany. Organohalogen Compounds, 73, 428-431.

Law K, Halldorson T, Danell R, Stern G, Gewurtz S, Alaee M, Marvin C, Whittle M and Tomy G (2006). Bioaccumulation and trophic transfer of some brominated flame retardants in a Lake Winnipeg (Canada) food web. Environmental Toxicology and Chemistry, 25(8), 2177-2186.

Law RJ, Losada S, Barber JL, Bersuder P, Deaville R, Brownlow A, Penrose R and Jepson PD (2013). Alternative flame retardants, Dechlorane Plus and BDEs in the blubber of harbour porpoises (*Phocoena phocoena*) stranded or bycaught in the UK during 2008. Environment International, 60, 81–88.

Li Y, Yu L, Zhu Z, Dai J, Mai B, Wu J and Wang J (2013a). Accumulation and effects of 90-day oral exposure to Dechlorane Plus in quail (*Coturnix coturnix*). Environ Toxicol Chem, 32(7), 1649-54.

Li Y, Yu L, Zhu Z, Wang J, Wu J, Mai B and Dai J (2013b). Accumulation pattern of Dechlorane Plus and associated biological effects on rats after 90 days of exposure. Chemosphere, 90, 2149-2156.

Li H, Zhang B, Wei Y, Wang F, Lydy MJ and You J (2014a). Bioaccumulation of highly hydrophobic organohalogen flame retardants from sediments: Application of toxicokinetics and passive sampling techniques. Environmental Science and Technology, 48, 6957-6964.

Li L, Wang W, Lv Q, Ben Y and Li X (2014b). Bioavailability and tissue distribution of Dechloranes in wild frogs (*Rana limnocharis*) from an e-waste recycling area in southeast China. Journal of Environmental Sciences, 26 (3), 636-642.

Liang X, Li W, Martyniuk CJ, Zha J, Wang Z, Cheng G and Giesy JP (2014). Effects of dechlorane

plus on the hepatic proteome of juvenile Chinese sturgeon (*Acipenser sinensis*). Aquatic Toxicology, 148, 83–91.

Liu W, Ye J and Jin M (2009). Enantioselective phytoeffects of chiral pesticides. J. Agric. Food Chem., 57, 2087–2095.

Ma Y, Xie Z, Halsall C, Möller A, Yang H, Zhong G, Cai M and Ebinghaus R (2015).The spatial distribution of organochlorine pesticides and halogenated flame retardants in the surface sediments of an Arctic fjord: The influence of ocean currents vs. glacial runoff. Chemosphere, 119, 953–960.

Mackay D, Arnot JA, Gobas FAPC and Powell DE (2013). Mathematical relationships between metrics of chemical bioaccumulation in fish. Environmental Toxicology and Chemistry, 32, 1459-1466.

[MoE] Ministry of Environment (2013). Results of Eco-toxicity tests of chemicals conducted by Ministry of the Environment in Japan (- March 2013). Available online http://www.env.go.jp/chemi/sesaku/02e.pdf accessed January 2014

Mayo-Bean K (2012). ECOSAR v.1.1. The ECOSAR (ECOlogical Structure Activity Relationship) Class Program for Microsoft Windows. U.S. Environmental Protection Agency. Office for Chemical Safety and Pollution Prevention. June 19 2012.

Mo L, Wu J-P, Luo X-J, Sun Y-X, Zheng X-B, Zhang Q, Zou F-S and Mai B-X (2013). Dechlorane Plus flame retardant in kingfishers (*Alcedo atthis*) from an electronic waste recycling site and a reference site, South China: Influence of residue levels on the isomeric composition. Environmental Pollution, 174, 57-62.

Möller A, Xie Z, Sturm R and Ebinghaus R (2010). Large-scale distribution and transport of Dechlorane Plus in air and sea water of the Arctic to Antarctic. Environ. Sci. Technol., 44, 8977–8982.

Möller A, Xie Z, Cai M, Zhong G, Huang P, Cai M, Sturm R, He J and Ebinghaus R (2011). Polybrominated diphenyl ethers vs alternate brominated flame retardants and Dechloranes from East Asia to the Arctic. Environ. Sci. Technol., 45, 6793–6799.

Möller A, Xie Z, Cai M, Sturm R and Ebinghaus R (2012). Brominated flame retardants and Dechlorane Plus in the marine atmosphere from Southeast Asia toward Antarctica. Environ. Sci. Technol., 46, 3141−3148.

Mortelmans KE and Tanaka W (1980). *In vitro* microbial mutagenesis assay of Dechlorane Plus. SRI International, SRI Project LSC-8971, March 1980. Testing laboratory: SRI International, Menlo Park, California, USA. Report no.: LSC-8971. Owner company: Hooker Chemical Corporation, Hooker Research Center. Report date: 1980-02-29.

Muir DCG, Teixeira C, de Jourdan B, Morris A, Backus S, Tomy G, Marvin C, MacInnis G, Spencer C and De Silva A (2014). Using Remote Environments to Detect New Persistent and Bioaccumulative Chemicals in Commerce. Chemicals Management Plan Environment and Climate Change Canada poster, presented September 2014. [As cited in Government of Canada, 2016.]

Muñoz-Arnanz J, Sáez M, Pacepavicius G, Alaee M and Jiménez B (2010). Dechlorane Plus and possible environmental metabolites in white stork eggs. Organohalogen Compd., 72, 933–936.

Muñoz-Arnanz J, Sáez M, Hiraldo F, Baos R, Pacepavicius MA, Jiménez B (2011). Dechlorane Plus and possible degradation products in white stork eggs from Spain. Environ. Int., 37(7), 1164-1168.

Muñoz-Arnanz J, Roscales JL, Vicente A, Aguirre JI and Jiménez B (2012). Dechlorane plus in eggs of two gull species (*Larus michahellis* and *Larus audouinii*) from the southwestern Mediterranean Sea. Anal. Bioanalytical Chem, 404 (9), 2765−2773.

Na G, Wei W, Zhou S, Gao H, Ma X, Qiu L, Ge L, Bao C and Yao Z (2015). Distribution characteristics and indicator significance of Dechloranes in multi-matrices at Ny-Ålesund in the Arctic. Journal of Environmental Sciences, 28, 8-13.

Newton S, Bidleman T, Bergknut M, Racine J, Laudon H, Gieslerf R and Wiberg K (2014). Atmospheric deposition of persistent organic pollutants and chemicals of emerging concern at two sites in northern Sweden. Environ. Sci.: Processes Impacts, 16, 298-305.

NITE (undated). Japan CHEmicals Collaborative Knowledge (J-CHECK) Database. National Institute of Tehcnology and Evaluation, Japan. http://www.safe.nite.go.jp/jcheck//DetailAction?cno=13560-89-9&mno=4-

0296&request_locale=en. Website accessed 10/04/2014.
Noyes PD, Haggard DE, Gonnerman GD and Tanguay RL (2015). Advanced morphological-behavioral test platform reveals neurodevelopmental defects in embryonic zebrafish exposed to comprehensive suite of halogenated and organophosphate flame retardants. Toxicol. Sci., 145, 177-195.
Occidental (2013). Product stewardship summary for Dechlorane Plus. Prepared: 1 December 2009, revised: 19 February 2013. Occidental Chemical Corporation. Available at: https://final-test.oxy.com/OurBusinesses/Chemicals/ResponsibleCare/documents/Dechlorane%20Plus.pdf [accessed 6 June 2014].
Occidental Chemical Company (2003). SIDS robust summaries (in IUCLID format), 201-15635, Substance ID: 13560-89-9. 16th December 2003. Available at http://www.epa.gov/hpv/pubs/summaries/dechlorp/c15635rs.pdf.
OECD (2009). OECD Series on Emission Scenario Documents Number 3: Emission Scenario Document on Plastic Additives. ENV/JM/MONO(2004)8/REV1, 9 July 2009.
Okonski K, Degrendele C, Melymuk L, Landlová L, Kukučka P, Vojta S, Kohoutek J, Čupr P and Klánová J (2014). Particle size distribution of halogenated flame retardants and implications for atmospheric deposition and transport. Environmental Science and Technology, 48 (24), 14426-14434.
O'Neil MJ (2013). *The Merck Index: An encyclopaedia of chemicals, drugs and biological,* 15th Edition, Royal Society of Chemistry.
Oscarson ET and Marias AJ (1975). Report to Hooker Chemical Corporation: 90-day subacute oral toxicity study with Dechlorane Plus 25 in albino rats. Industrial Bio-Test Laboratories Inc., IBT No. 622-06273, 29 September 1975. Testing laboratory: Industrial Bio-Test Laboratories Ind., Northbrook, Illinois, USA. Report no.: IBT No. 622-06273. Owner company: Hooker Chemical Corporation. Report date: 1975-09-29.
OxyChem (2007). Dechlorane Plus Manual, Ver: 7-27-07, published online at http://www.oxy.com/OurBusinesses/Chemicals/Products/Documents/dechloraneplus/dechlorane_plus.pdf.
Peng H, Zhang K, Wan Y and Hu J (2012). Tissue distribution, maternal transfer, and age-related accumulation of Dechloranes in Chinese Sturgeon. Environ. Sci. Technol., 46, 9907−9913.
Peng H, Wan Y, Zhang K, Sun J and Hu J (2014). Trophic transfer of Dechloranes in the marine food web of Liaodong Bay, north China. Environ. Sci. Technol., 48, 5458−5466.
Peng Y, Wu J-P, Tao L, Mo L, Zheng X-B, Tang B, Luo X-J and Mai B-X (2015). Accumulation of Dechlorane Plus flame retardant in terrestrial passerines from a nature reserve in South China: The influences of biological and chemical variables. Science of the Total Environment, 514, 77-82.
Plastics Europe (undated). PESTool online, available online at http://www.plasticseurope.org/plasticssustainability/consumer-protection/reach.aspx
Qiu XH, Marvin CH and Hites RA (2007). Dechlorane Plus and other flame retardants in a sediment core from Lake Ontario. Environ. Sci. Technol., 41, 6014–6019.
Qiu X, Venier M and Hites RA (2008). Dechlorane Plus: a review with new data. Organohalogen Compounds, 70, 216-219.
Qiu XH and Hites RA (2008). Dechlorane Plus and other flame retardants in tree bark from the northeastern United States. Environ. Sci. Technol., 42, 31–36.
Ren NQ, Sverko E, Li YF, Zhang Z, Harner T, Wang DG, Wan XN and McCarry BE (2008). Levels and isomer profiles of Dechlorane Plus in Chinese air. Environ. Sci. Technol., 42, 6476–6480.
Ren G, Yu Z, Ma S, Li H, Peng P, Sheng G and Fu J (2009). Determination of dechlorane plus in serum from electronics dismantling workers in China. Environmental Science and Technology, 43, 9453–9457.
Ren G, Wang Z, Yu Z, Wang Y, Ma S, Wu M, Sheng G and Fu J (2013). Primary investigation on contamination pattern of legacy and emerging halogenated organic pollutions in freshwater fish from Liaohe River, Northeast China. Environmental Pollution, 172, 94-99.
Rjabova J, Bartkevics V, Zacs D (2016). The occurrence of Dechlorane Plus and related norbornene-based flame retardants in Baltic wild salmon (*Salmo salar*). Chemosphere, 147, 210-217.
Russell M (1973). Report to Hooker Chemical Corporation, Subsidiary of Occidental Petroleum

Corporation: Four-day dynamic fish toxicity study with Dechlorane Plus 515 in bluegills. Industrial Bio-Test laboratories, IBT No. R2488, 17 January 1973. Testing laboratory: Industrial Bio-Test Laboratories. Report no.: IBT No. R2488. Owner company: Hooker Chemical Corporation. Report date: 1973-01-16.

Sahlström LMO, Sellström U, De Wit CA, Lignell S and Darnerud PO (2014). Brominated flame retardants in matched serum samples from Swedish Shanghai mothers and their toddlers. Environmental Science and Technology, 48 (13), 7584-7592.

Salamova A and Hites R (2013). Brominated and chlorinated flame retardants in tree bark from around the globe. Environmental Science and Technology, 47, 349-354.

Salamova A, Hermanson MH and Hites RA (2014). Organophosphate and halogenated flame retardants in atmospheric particles from a European Arctic site. Environ. Sci. Technol., 48, 6133−6140.

Santín G, Barón E, Eljarrat E and Barceló D (2013). Emerging and historical halogenated flame retardants in fish samples from Iberian rivers. Journal of Hazardous Materials, 263, 116-121.

Scharf DJ (1978). [Inhouse] Monthly Status Report, dated February 13, 1978.

Schlabach M, Remberger M, Brorström-Lundén E, Norström K, Kaj L, Andersson H, Herzke D, Borgen A and Harju M (2011). Brominated Flame Retardants (BFR) in the Nordic Environment. TemaNord 2011:528. Nordic Council of Ministers, Copenhagen.

Schlabach M, van Bavel B, Lomba JAB, Borgen A, Fjeld E, Halse A-K, Nikiforov V, Nizzetto PB, Reid M, Rostkowski P and Vogelsang C (2017). Screening programme 2016 - Selected compounds with relevance for EU regulation. NILU - Norwegian Institute for Air Research NILU report 34/2017, no. M818.

Schroeder RE (2008). Dechlorane Plus: oral repeat dose toxicity and reproduction/developmental toxicity screening study in rats. MPI Research Inc., Study Number 786-025, 26 September 2008. Testing laboratory: MPI Research Inc., Mattawan, Michigan, USA. Report no.: 786-025. Report date: 2008-09-25.

She Y-Z, Wu J-P, Zhang Y, Peng Y, Mo L, Luo X-J and Mai B-X (2013). Bioaccumulation of polybrominated diphenyl ethers and several alternative halogenated flame retardants in a small herbivorous food chain. Environmental Pollution, 174, 164-170.

Shen L, Reiner EJ, MacPherson K, Kolic TM, Sverko E, Helm P, Bhavsar S, Brindle ID and Marvin CH (2010). Identification and screening analysis of halogenated norbornene flame retardants in the Laurentian Great Lakes: Dechloranes 602, 603, and 604. Environ. Sci. Technol., 44, 760–766.

Shen L, Reiner EJ, Helm PA, Marvin CH, Hill B, Zhang X, MacPherson KA, Kolic TM, Tomy GT and Brindle ID (2011a). Historic trends of Dechloranes 602, 603, 604, Dechlorane Plus and other norbornene derivatives and their bioaccumulation potential in Lake Ontario. Environ. Sci. Technol., 45, 3333–3340.

Shen L, Reiner EJ, MacPherson KA, Kolic TM, Helm PA, Richman LA, Marvin CH, Burniston DA, Hill B, Brindle ID, McCrindle R and Chittim BG (2011b). Dechloranes 602, 603, 604, dechlorane plus, and chlordene plus, a newly detected analogue, in tributary sediments of the Laurentian Great Lakes. Environ. Sci. Technol. 45, 693– 699.

Shen L, Jobst KJ, Helm PA, Reiner EJ, McCrindle R, Tomy GT, Backus S, Brindle ID and Marvin CH (2012). Identification and determination of the dechlorination products of Dechlorane 602 in Great Lakes fish and Arctic beluga whales by gas chromatography-high resolution mass spectrometry. Anal. Bioanal. Chem., 404, 2737–2748.

Siddique S, Xian QM, Abdelouahab N, Takser L, Phillips SP, Feng YL, Wang B and Zhu JP (2012). Levels of dechlorane plus and polybrominated diphenylethers in human milk in two Canadian cities. Environ Int., 39, 50−55.

Spehar RL, Veith GD, DeFoe DL and Bergstedt BV (1979). Toxicity and bioaccumulation of hexachlorocyclopentadiene, hexachloronorbornadiene and heptachloronorbornene in larval and early juvenile fathead minnows, *Pimephales promelas*. Bulletin of Environmental Contamination and Toxicology, 21, 576-583.

Strid A, Bruhn C, Sverko E, Svavarsson J, Tomy G and Bergman Å (2013). Brominated and chlorinated flame retardants in liver of Greenland shark (*Somniosus microcephalus*). Chemosphere, 91, 222–228.

Su G, Letcher RJ, Moore JN, Williams LL, Martin PA, de Solla SR and Bowerman WW (2015). Spatial and temporal comparisons of legacy and emerging flame retardants in herring gull eggs from colonies spanning the Laurentian Great Lakes of Canada and United States. Environmental Research, 142, 720-730.

Sühring R, Möller A, Freese M, Pohlmann J, Wolschke H, Sturm R, Xie Z, Hanel R and Ebinghaus R, (2013). Brominated flame retardants and dechloranes in eels from German rivers. Chemosphere 90, 118–124.

Sühring R, Byer J, Freese M, Pohlmann J-D, Wolschke H, Möller A, Hodson PV, Alaee M, Hanel R and Ebinghaus R (2014). Brominated flame retardants and Dechloranes in European and American eels from glass to silver life stages. Chemosphere, 116, 104-111.

Sühring R, Freese M, Schneider M, Schubert S, Pohlmann JD, Alaee M, Wolschke H, Hanel R, Ebinghaus R and Marohn L (2015). Maternal transfer of emerging brominated and chlorinated flame retardants in European eels. Science of the Total Environment, 530-531, 209-218.

Sun Y, Luo X, Wu J, Mo L, Chen S, Zhang Q, Zou F and Mai B (2012). Species- and tissue-specific accumulation of Dechlorane Plus in three terrestrial passerine bird species from the Pearl River Delta, South China. Chemosphere, 89, 445–451.

Sun Y-X, Xu X-R, Hao Q, Luo X-J, Ruan W, Zhang Z-W, Zhang Q, Zou F-S and Mai B-X (2014). Species-specific accumulation of halogenated flame retardants in eggs of terrestrial birds from an ecological station in the Pearl River Delta, South China. Chemosphere, 95, 442–447.

Sun Y-X, Zhang Z-W, Xu X-R, Hu Y-X, Luo X-J, Cai M-G and Mai B-X (2015). Bioaccumulation and biomagnification of halogenated organic pollutants in mangrove biota from the Pearl River Estuary, South China. Marine Pollution Bulletin, 99, 150-156.

Sverko E, Tomy GT, Marvin CH, Zaruk D, Reiner E, Helm PA, Hill B and McCarry BE (2008). Dechlorane Plus levels in sediment of the lower Great Lakes. Environ. Sci. Technol., 42, 361–366.

Sverko E, Harner T, Lee SC and McCarry BE (2010a). Dechlorane Plus in the Global Atmospheric Passive Sampling (GAPS) study. Presented at Dioxin, San Antonio, TX, September 12-17 2010; #1505. [As cited by Sverko *et al*. (2011) and Xian *et al*., 2011[39]]

Sverko E, Reiner EJ, Tomy GT, McCrindle R, Shen L, Arsenault G, Zaruk D, Macpherson KA, Marvin CH, Helm PA and McCarry BE (2010b). Compounds structurally related to dechlorane plus in sediment and biota from Lake Ontario (Canada). Environ. Sci. Technol., 44, 574–579.

Sverko E, Tomy GT, Palace V, Smith LAP and McCarry BE (2010c). Fate of related compounds to Dechlorane Plus in a Lake Ontario (Canada) food web. Presented at Dioxin, San Antonio, TX, September 12-17, 2010; #1504. [As cited by Sverko *et al*., 2011]

Sverko E, Tomy GT, Reiner EJ, Li Y-F, McCarry BE, Arnot JA, Law RJ and Hites RA (2011). Dechlorane Plus and related compounds in the environment: a review. Environ. Sci. Technol., 45, 5088–5098.

Tao W, Zhou Z, Shen L and Zhao B (2015). Determination of dechlorane flame retardants in soil and fish at Guiyu, an electronic waste recycling site in south China. Environmental Pollution, 206, 361-368.

Thanh TL, Yoon H-W, Kim J-C and Chang Y-S (2013). Assessment of the potential risks to microbiota from low dose Dechlorane Plus exposure in the soil mimic condition. Organohalogen Compounds, 75, 328-331. http://www.dioxin20xx.org/pdfs/2013/140.pdf

Tomy GT, Palace VP, Halldorson THJ, Braekevelt E, Danell RW, Evans B, Brinkworth L and Fisk AT (2004). Bioaccumulation, biotransformation and biochemical effects of brominated diphenyl ethers (BDEs) in juvenile lake trout (*Salvelinus namaycush*). Environ. Sci. Technol., 38, 1496–1504.

Tomy GT, Budakowski WR, Halldorson THJ, Whittle DM, Keir MJ, Marvin C, MacInnis G and Alaee M (2004). Biomagnification of α- and γ-hexabromocyclododecane isomers in a Lake Ontario food web. Environmental Science and Technology, 38, 2298-2303.

[39] This appears to have been a poster, previously downloadable from http://www.xcdtech.com/dioxin2010/pdf/1505.pdf. However, this link no longer works, and the www.dioxin20xx.org/ website does not have any further information.

Tomy GT, Pleskach K, Ismail N, Whittle DM, Helm PA, Sverko E, Zaruk D and Marvin CH (2007). Isomers of Dechlorane Plus in Lake Winnipeg and Lake Ontario food webs. Environ. Sci. Technol., 41, 2249–2254.

Tomy GT, Thomas CR, Zidane TM, Murison KE, Pleskach K, Hare J, Arsenault G, Marvin CH and Sverko E (2008). Examination of isomer specific bioaccumulation parameters and potential *in vivo* hepatic metabolites of syn- and anti-Dechlorane Plus isomers in juvenile rainbow trout (*Oncorhynchus mykiss*).Environ Sci Technol., 42(15), 5562-7.

Tomy GT, Sverko E, Palace V, Rosenberg B, McCrindle R, McAlees A, Smith LAP, Byer J, Pacepavicius G, Alaee M and McCarry BE (2013). Dechlorane plus monoadducts in a Lake Ontario (Canada) food web and biotransformation by lake trout (*Salvelinus namaycush)* liver microsomes. Environmental Toxicology and Chemistry, 32, 1376–1381.

Trzyna GE and Paa H (1975). Report to Hooker Chemical Corporation: 28-day subacute dermal toxicity study with EC number: 236-948-91,6,7,8,9,14,15,16,17,17,18,18-dodecachloropentacyclo[12.2.1.16,9.02,13.05,10]oc. CAS number: 13560-89-9 2013-05-15 CSR-PI-5.4.1 CHEMICAL SAFETY REPORT 64 Dechlorane 25 in albino rabbits. Industrial Bio-Test Laboratories Inc., IBT No. 601-06280, 13 June 1975. Testing laboratory: Industrial Bio-Test Laboratories Inc., Northbrook, Illinois, USA. Report no.: IBT No. 601- 06280. Owner company: Hooker Chemical Corporation. Report date: 1975-06-13.

UNEP [United Nations Environment Programme] (1998). Persistent Organic Pollutants: an assessment report on: DDT-Aldrin-Dieldrin-Endrin-Chlordane-Heptachlor-Hexachlorobenzene-Mirex-Toxaphene-Polychlorinated Biphenyls-Dioxins and Furans. UNEP/POPS/INC.1/INF/10 15th June 1998

UNEP (2009). Report of the Persistent Organic Pollutants Review Committee on the work of its fifth meeting. Addendum: Risk profile on endosulfan. UNEP/POPS/POPRC.5/10/Add.2 13th December 2009

United Nations (2001). Annex A of the *Stockholm Convention of Persistent Organic Pollutants,* 43 pp; Amended (Geneva, 2009) 63 pp, 2001.

U.S. EPA [United States Environmental Protection Agency] (1977). A Rapid Assessment of the Toxicity of Three Chlorinated Cyclodiene Insecticide Intermediates to Fathead Minnows. Environmental Research Laboratory, Duluth, Minnesota, USA. Ecological Research Series. EPA-600/3-77-099. August 1977.

U.S. EPA (2011). Screening-level hazard characterization, Dechlorane Plus (CASRN 13560-89-9). U.S Environmental Protection Agency, Hazard Characterisation Department, March 2011.

U.S. EPA (2012). Estimation Programs Interface Suite™ for Microsoft® Windows, v4.11. U.S. Environmental Protection Agency, Washington, DC, USA. Available online at http://www.epa.gov/opptintr/exposure/pubs/episuitedl.htm as of November 2012.

U.S. EPA (2014). An alternatives assessment for the flame retardant decabromodiphenyl ether (DecaBDE). Available at: https://www.epa.gov/sites/production/files/2014-05/documents/decabde_final.pdf

Venier M, Wierda M, Bowerman WW and Hites RA (2010). Flame retardants and organochlorine pollutants in bald eagle plasma from the Great Lakes region. Chemosphere, 80, 1234–1240.

Venier M and Hites RA (2011). Flame retardants in the serum of pet dogs and in their food. Environ. Sci. Technol., 45, 4602–4608.

Vorkamp K and Rigét FF (2014). A review of new and current-use contaminants in the Arctic environment: Evidence of long-range transport and indications of bioaccumulation. Chemosphere, 111, 379–395.

Vorkamp K, Bossi R, Riget FF, Skov H, Sonne C and Dietz R (2015). Novel brominated flame retardants and Dechlorane Plus in Greenland air and biota. Environmental Pollution, 196, 284-291.

Walker LA, Moeckel C, Pereira MG, Potter ED, Chadwick EA and Shore RF (2013). Liver concentrations of flame retardants in Eurasian otters (*Luta lutra*) collected from Britain in 2010 & 2011: a Predatory Bird Monitoring Scheme (PBMS) Report. Centre for Ecology & Hydrology, Lancaster, UK.

Wang DG, Yang M, Qi H, Sverko E, Ma WL, Li YF, Alaee M, Reiner EJ and Shen L (2010). An Asia-specific source of Dechlorane Plus: Concentration, isomer profiles, and other related compounds. Environ. Sci. Technol., 44, 6608–6613.

Wang J, Tian M, Chen SJ, Zheng J, Luo XJ, An TC and Mai BX (2011). Dechlorane Plus in house

dust from e-waste recycling and urban areas in South China: sources, degradation, and human exposure. Environmental Toxicology and Chemistry, 30, 1965–1972.

Wang L, Jia H, Liu X, Yang M, Hong W, Sun Y, Su Y, Qi H, Song W, Lin J and Y-F Li (2012). Dechloranes in a river in northeastern China: Spatial trends in multi-matrices and bioaccumulation in fish (*Enchelyopus elongatus*). Ecotoxicology and Environmental Safety, 84, 262–267.

Wang D-G, Alaee M, Byer JD, Brimble S and Pacepavicius G (2013a). Human health risk assessment of occupational and residential exposures to dechlorane plus in the manufacturing facility area in China and comparison with e-waste recycling site. Science of the Total Environment, 445–446, 329–336.

Wang S, Huang J, Yang Y, Yu G, Deng S and Wang B (2013b). Photodegradation of Dechlorane Plus in n-nonane under the irradiation of xenon lamp. Journal of Hazardous Materials, 260, 16– 23.

Wang Y, Xu M, Jin J, He S, Li M and Sun Y (2014). Concentrations and relationships between classes of persistent halogenated organic compounds in pooled human serum samples and air from Laizhou Bay, China. Science of the Total Environment, 482–483, 276–282.

Wang D-G, Guo M-X, Pei W, Byer JD and Wang Z (2015). Trophic magnification of chlorinated flame retardants and their dechlorinated analogs in a fresh water food web. Chemosphere, 118, 293–300.

Wania F (2006). Potential of degradable organic chemicals for absolute and relative enrichment in the Arctic. Environ. Sci. Technol., 40, 569–577.

Wegmann F, Cavin L, MacLeod M, Scheringer M and Hungerbühler K (2009). The OECD software tool for screening chemicals for persistence and long-range transport potential. Environmental Modeling and Software, 24, 228-237.

WHO [World Health Organisation] (1984a). International Programme On Chemical Safety Environmental Health Criteria 38 Heptachlor. Geneva.

WHO (1984b). International Programme On Chemical Safety Environmental Health Criteria 44 Mirex. Geneva.

WHO (1984c). International Programme On Chemical Safety Environmental Health Criteria 34 Chlordane. Geneva.

WHO (1989). International Programme On Chemical Safety Environmental Health Criteria 91 aldrin and dieldrin. Geneva.

WHO (1992). International Programme On Chemical Safety Environmental Health Criteria 130 Endrin. Geneva.

WHO (1995). A review of selected persistent organic pollutants DDT-Aldrin-Dieldrin-Endrin-Chlordane, Heptachlor- Hexachlorobenzene- Mïrex- Toxaphene, Polychlorinated biphenyls, Dioxins and Furans. PCS/95.39.

WHO (1996). International Programme on Chemical Safety Environmental Health Criteria 185 Chlorendic Acid and Anhydride. Geneva.

Wolschke H, Meng X-Z, Xie Z, Ebinghaus R, Cai M (2015). Novel flame retardants (N-FRs), polybrominated diphenyl ethers (PBDEs) and dioxin-like polychlorinated biphenyls (DL-PCBs) in fish, penguin, and skua from King George Island, Antarctica. Mar. Pollut. Bull., 96, 513-518.

Wu JP, Zhang Y, Luo XJ, Wang J, Chen SJ, Guan YT and Mai BX (2010). Isomer-specific bioaccumulation and trophic transfer of Dechlorane Plus in the freshwater food web from a highly contaminated site, South China. Environ. Sci. Technol., 44(2), 606-611.

Wu B, Liu S, Guo X, Zhang Y, Zhang X, Li M and Cheng S (2012). Responses of mouse liver to dechlorane plus exposure by integrative transcriptomic and metabonomic studies. Environ. Sci. Technol., 46, 10758–10764.

Wu J, She Y, Zhang Y, Peng Y, Mo L, Luo X and Mai B (2013). Sex-dependent accumulation and maternal transfer of dechlorane plus flame retardant in fish from an electronic waste recycling site in South China. Environmental Pollution, 177, 150-155.

Xian Q, Siddique S, Li T, Feng Y-L, Takser L and Zhu J (2011). Sources and environmental behavior of dechlorane plus – A review. Environment International, 37, 1273–1284.

Xiao H, Shen L, Yushan S, Barresi E, DeJong M, Hung H, Lei Y, Wania F, Reiner E, Sverko E and Kang S (2012). Atmospheric concentrations of halogenated flame retardants at two remote locations: the Canadian High Arctic and the Tibetan Plateau. Environmental Pollution, 161,

154-161.
Xiao R, Adolfsson-Erici M, Akerman G, McLachlan MS and MacLeod M (2013). A benchmarking method to measure dietary absorption efficiency of chemicals by fish. Environ. Toxicol. Chem., 32, 2695-2700.
Yan X, Zheng J, Chen KH, Yang J, Luo XJ, Yu LH, Chen SJ, Mai BX and Yang ZY (2012). Dechlorane Plus in serum from e-waste recycling workers: influence of gender and potential isomer-specific metabolism. Environ. Int., 49, 31–37.
Yang M, Jia H, Ma W, Qi H, Cui S and Li Y (2012). Levels, compositions, and gas-particle partitioning of polybrominated diphenyl ethers and dechlorane plus in air in a Chinese northeastern city. Atmospheric Environment, 55, 73-79.
Yang R, Zhang S, Li X, Luo D and Jing C (2016). Dechloranes in lichens from the southeast Tibetan Plateau: Evidence of long-range atmospheric transport. Chemosphere, 144, 446-451.
Yu L-H, Luo X-J, Wu J-P, Liu L-Y, Song J, Sun Q-H, Zhang X-L, Chen D and Mai B-X (2011). Biomagnification of higher brominated PBDE congeners in an urban terrestrial food web in North China based on field observation of prey deliveries. Environmental Science & Technology, 45, 5125–5131.
Yu L, Luo X, Zheng X, Zeng Y, Chen D, Wu J and Mai B (2013). Occurrence and biomagnification of organohalogen pollutants in two terrestrial predatory food chains. Chemosphere, 93, 506–511.
Yu L-H, Luo X-J, Liu H-Y, Zeng Y-H, Zheng X-B, Wu J-P, Yu Y-J and B-X Mai (2014). Organohalogen contamination in passerine birds from three metropolises in China: Geographical variation and its implication for anthropogenic effects on urban environments. Environmental Pollution, 188, 118-123.
Zeng Y-H, Luo X-J, Tang B, Zheng X-B and Mai B-X (2014a). Gastrointestinal absorption, dynamic tissue-specific accumulation, and isomer composition of dechlorane plus and related analogs in common carp by dietary exposure. Ecotoxicology and Environmental Safety, 100, 32–38.
Zeng Y-H, Luo X-J, Zheng X-B, Tang B, Wu J-P and Mai B-X (2014b). Species-specific bioaccumulation of halogenated organic pollutants and their metabolites in fish serum from an e-waste site, South China. Archives of Environmental Contamination and Toxicology, 67 (3), 348-357.
Zhang K, Wan Y, An LH and Hu JY (2010a). Trophodynamics of polybrominated diphenyl ethers and methoxylated polybrominated diphenyl ethers in a marine food web. Environ. Toxicol. Chem., 29, 2792−2799.
Zhang Y, Luo XJ, Wu JP, Liu J, Wang J, Chen SJ and Mai BX (2010b). Contaminant pattern and bioaccumulation of legacy and emerging organhalogen pollutants in the aquatic biota from an e-waste recycling region in South China. Environmental Toxicology and Chemistry, 29, 852–859.
Zhang Y, Wu J-P, Luo X-J, Wang J, Chen S-J and Mai B-X (2011a). Tissue distribution of Dechlorane Plus and its dechlorinated analogs in contaminated fish: High affinity to the brain for anti-DP. Environmental Pollution, 159. 3647-3652.
Zhang X-L, Luo X-J, Liu H-Y, Yu L-H, Chen S-J and Mai B-X (2011b). Brominated flame retardants and Dechlorane Plus in waterbirds from an e-waste recycling region in South China: associated with trophic level and diet sources. Environ. Sci. Technol., 45, 400–405.
Zhang Y, Wu J-P, Luo X-J, Sun Y-X, Mo L, Chen S-J and Mai B-X (2011c). Biota-sediment accumulation factors for Dechlorane Plus in bottom fish from an electronic waste recycling site, South China. Environment International, 37, 1357–1361.
Zhang K, Wan Y, Jones PD, Wiseman S, Giesy JP and Hu JY (2012). Occurrences and fates of hydroxylated polybrominated diphenyl ethers in marine sediments in relation to trophodynamics. Environ. Sci. Technol., 46, 2148−2155.
Zhang H, Wang P, Li Y, Shang H, Wang Y, Wang T, Zhang Q and Jiang G (2013). Assessment on the occupational exposure of manufacturing workers to Dechlorane Plus through blood and hair analysis. Environ. Sci. Technol., 47, 10567−10573.
Zhang L, Ji F, Li M, Cui Y and Wu B (2014). Short-term effects of Dechlorane Plus on the earthworm *Eisenia fetida* determined by a systems biology approach. Journal of Hazardous Materials, 273, 239–246.

Zhang Y, Luo XJ, Mo L, Wu JP, Mai BX and Peng YH (2015). Bioaccumulation and translocation of polyhalogenated compounds in rice (*Oryza sativa* L.) planted in paddy soil collected from an electronic waste recycling site, South China. Chemosphere, 137, 25-32.

Zhao L, Gong N, Mi D, Luan C, Shao K, Jia H and Sun Y (2014). Kinetics of stereoselective enrichment of Dechlorane Plus in *Ulva pertusa*. Chemosphere, 111, 580-586.

Zheng J, Wang J, Luo XJ, Tian M, He LY, Yuan JG, Mai BX and Yang ZY (2010). Dechlorane Plus in human hair from an e-waste recycling area in south China: Comparison with dust. Environ. Sci. Technol., 44 (24), 9298−9303.

Zheng X-B, Wu J-P, Luo X-J, Zeng Y-H, She Y-Z and Mai B-X (2012). Halogenated flame retardants in home-produced eggs from an electronic waste recycling region in South China: Levels, composition profiles, and human dietary exposure assessment. Environment International, 45, 122–128.

Zheng, X-B, Luo X-J, Zeng Y-H, Wu J-P and Mai B-X (2014a). Sources, gastrointestinal absorption and stereo-selective and tissue-specific accumulation of Dechlorane Plus (DP) in chicken. Chemosphere, 114, 241-246.

Zheng X-B, Luo X-J, Zeng Y-H, Wu J-P, Chen S-J and Mai B-X (2014b). Halogenated flame retardants during egg formation and chicken embryo development: Maternal transfer, possible biotransformation, and tissue distribution. Environmental Toxicology and Chemistry, 33 (8), 1712-1719.

Zhou S N, Siddique S, Lavoie L, Takser L, Abdelouahab N and Zhu J (2014). Hexachloronorbornene-based flame retardants in humans: Levels in maternal serum and milk. Environment International, 66, 11–17.

Zhu B, Lai NLS, Wai T-C, Leo L. Chan LL, Lam JCW and Lam PKS (2014). Changes of accumulation profiles from PBDEs to brominated and chlorinated alternatives in marine mammals from the South China Sea. Environment International, 66, 65–70.

Zitko V (1980). The uptake and excretion of mirex and dechloranes by juvenile atlantic salmon. Chemosphere, 9, 73-78.

Appendix 1 Detailed summaries of data cited in the main report

Contents

APPENDIX 1 ..... DETAILED SUMMARIES OF DATA CITED IN THE MAIN REPORT 86

1 IDENTITY OF THE SUBSTANCE AND PHYSICAL AND CHEMICAL PROPERTIES 88

2 CLASSIFICATION AND LABELLING 88

3 ENVIRONMENTAL FATE PROPERTIES 88

3.1 DEGRADATION 88
3.1.1 Abiotic degradation 88
3.1.2 Biodegradation 90
3.2 ENVIRONMENTAL DISTRIBUTION 96
3.2.1 Adsorption/desorption 96
3.2.2 Volatilisation 97
3.3 POTENTIAL FOR LONG RANGE TRANSPORT 97
3.4 BIOACCUMULATION 103
3.4.1 Bioaccumulation in aquatic organisms (pelagic and sediment organisms) 103
3.4.2 Bioaccumulation in terrestrial organisms (soil-dwelling organisms, vertebrates) 147

4 HUMAN HEALTH HAZARD ASSESSMENT RELEVANT FOR THE PBT ASSESSMENT 163

4.1 TOXICOKINETICS 163
4.2 REPEATED DOSE TOXICITY 163
4.2.1 Repeated dose toxicity: oral 163
4.2.2 Repeated dose toxicity: inhalation 164
4.2.3 Repeated dose toxicity: dermal 164
4.2.4 Summary and discussion of repeated dose toxicity 164
4.3 MUTAGENICITY 165
4.3.1 In vitro data 165
4.3.2 In vivo data 165
4.3.3 Summary and discussion of mutagenicity 165
4.4 CARCINOGENICITY 166
4.4.1 Summary and discussion of carcinogenicity 166
4.5 TOXICITY TO REPRODUCTION 166
4.5.1 Effects on fertility and development 166
4.5.2 Summary and discussion of toxicity to reproduction 166
4.6 SUMMARY OF MAMMALIAN TOXICITY 166

5 ENVIRONMENTAL HAZARD ASSESSMENT 167

5.1 AQUATIC COMPARTMENT (INCLUDING SEDIMENT) 167
5.1.1 Pelagic toxicity 167
5.1.2 Sediment organisms 180
5.2 TERRESTRIAL COMPARTMENT 181
5.3 ATMOSPHERIC COMPARTMENT 185
5.4 MICROBIOLOGICAL ACTIVITY IN SEWAGE TREATMENT SYSTEMS 185
5.4.1 Toxicity to aquatic micro-organisms 185
5.5 NON COMPARTMENT SPECIFIC EFFECTS RELEVANT FOR THE FOOD CHAIN (SECONDARY POISONING) 185
5.5.1 Toxicity to birds 185
5.5.2 Toxicity to mammals 188

6 MONITORING DATA 188

ANNEX LITERATURE SEARCH 189

APPENDIX 2 TRANSFORMATION PRODUCTS 196

APPENDIX 3 MEASURED CONCENTRATIONS OF DECHLORANE PLUS IN BIOTA ASSOCIATED WITH THE AQUATIC ENVIRONMENT 205

APPENDIX 4 MEASURED CONCENTRATIONS OF DECHLORANE PLUS IN BIOTA ASSOCIATED WITH THE TERRESTRIAL ENVIRONMENT 233

APPENDIX 5 OTHER EUROPEAN MONITORING STUDIES 246

APPENDIX 6 COMPARISON OF LABORATORY BIOCONCENTRATION DATA BETWEEN SUBSTANCES 250

APPENDIX 7 BENCHMARKING OF BIOACCUMULATION USING POLYCHLOROBIPHENYLS (PCBS) 256

APPENDIX 8 PREDICTED AQUATIC TOXICITY OF SELECTED DECHLORANE PLUS ANALOGUES USING EPISUITE V4.11 258

APPENDIX 9 FURTHER REFERENCES THAT APPEAR RELEVANT BUT ARE NOT INCLUDED IN THE ASSESSMENT 260

Tables

Table 1: Abiotic monitoring data for Dechlorane Plus in remote locations 99
Table 2: Concentrations of Dechlorane Plus in a Chinese marine food web (from Peng *et al.*, 2014) 131
Table 3: Concentrations of Dechlorane Plus and potential degradants in a Chinese freshwater food web (from Wang *et al.*, 2015) 135
Table 4: Concentrations of Dechlorane Plus in a Chilean marine food web (from Barón *et al.*, 2013) 137
Table 5: Dechlorane Plus concentrations in bird egg samples from Spain (from Barón *et al.*, 2014a) 152
Table 6: Summary of fish bioaccumulation data from WHO (1993) where uptake or depuration data are available 256

Figures

Figure 1: Regressions between trophic level and concentrations of syn- and anti- isomers in aquatic species at a contaminated site in South China (bars represent ± 1 standard error) 117
Figure 2: Regression between trophic level and concentrations of the anti- isomer in aquatic species in Lake Winnipeg (error bars represent ± 1 standard error) 122
Figure 3: Relationship between $\delta^{15}N$ and Dechlorane Plus muscle concentrations in wild birds from South China 128
Figure 4: Regressions between trophic level and concentrations of anti-Dechlorane Plus in aquatic species in a Chinese marine food web (error bars represent ± 1 standard error) 133
Figure 5: Regressions between trophic level and concentrations of Dechlorane Plus (anti- and total isomers) in aquatic species 135
Figure 6: Plot of mean total Dechlorane Plus concentrations in falconiform eggs versus $\delta^{15}N$ 153
Figure 7: Plot of mean Dechlorane Plus concentrations in passerine muscle versus $\delta^{15}N$ 155

The sections below are copied from the PBT factsheet for the substance discussed at the ECHA PBT Expert Group in 2016, although some have been updated. The key data for determining vPvB, as well as information such as *Identity of the substance and physical and chemical properties*, have been moved to the main SVHC dossier. However, to keep the main dossier readable, the studies that are of lower relevance for the conclusion are summarized in detail here instead.

1 IDENTITY OF THE SUBSTANCE AND PHYSICAL AND CHEMICAL PROPERTIES

2 CLASSIFICATION AND LABELLING

3 ENVIRONMENTAL FATE PROPERTIES

3.1 Degradation

3.1.1 Abiotic degradation

3.1.1.1 Hydrolysis

3.1.1.2 Oxidation

3.1.1.3 Phototransformation/photolysis

3.1.1.3.1 *Phototransformation in air*

No data are reported by the Registrant.

A UV-vis spectrum provided in the REACH registration dossier in the range 215 – 800 nm indicates that the substance has no significant absorbance above about 260 nm (the substance was dissolved in tetrahydrofuran, which is transparent to these wavelengths). The Registrant's technical manual indicates that Dechlorane Plus has "excellent UV stability" in comparison to two common brominated flame retardants used in ABS plastic (Oxychem, 2006).

Sverko *et al*. (2008) irradiated a 100 ng/mL [µg/L] solution of each isomer in isooctane to UV-A light (λ ≥ 365 nm) at 30 W and a distance of 20 cm for 24 h per day over 31 days. The parent substance concentration had decreased by 10 % after 168 h [7 d] with a further loss of 40 % at 264 h [11 d] and 65 % at 504 h [21 d]. Anti-DP was said to degrade more readily than the syn- isomer, although no information was provided on relative rates, and degradation products were not identified.

Wang *et al.* (2011) carried out some photodegradation experiments with syn- and anti-DP in isooctane. Solutions of the substance were exposed to UV light from a 125 W high pressure mercury lamp at 20 °C for up to 30 minutes. In both cases the concentration of Dechlorane Plus declined to around 3 % of its initial value after 15 minutes and the degradation products were mainly the reductive dechlorination products DP-1Cl and DP-2Cl. The half-life was determined to be 2.82 minutes for anti-DP and 2.94 minutes for syn-DP.

Li *et al*. (2013b) conducted three photolytic degradation experiments by exposing solutions of the anti- isomer, syn- isomer and a commercial product to UV light. The solvent and conditions are not described. In addition to anti- and syn-DP-1Cl, at least two unknown products were identified in all samples following exposure. The amounts were not quantified.

Wang *et al.* (2013b) carried out photolytic degradation studies with a commercial Dechlorane Plus product in n-nonane using various wavelength ranges between 200 and 750 nm (300 W xenon lamp). Photodegradation was found to occur with UV-light. The main transformation products were thought to be lower chlorinated dechloranes formed by reductive dechlorination processes (with 1 to 4 fewer chlorine atoms per molecule). No photoisomerisation products or solvent adducts were observed. The quantum yields for the reaction at wavelengths between 200-280 nm (UV-C range) were 2-3 orders of magnitude higher than measured at 280-320 nm (UV-B range). The corresponding degradation half-lives were around 3.2-4.1 minutes and 709-950 minutes, respectively. No quantum yields could be determined at wavelengths between 320 and 700 nm. The f_{anti} in the commercial product used was 0.75 and the f_{anti} did not change significantly during the degradation, implying that both syn- and anti-DP degraded at similar rates.

Tao *et al*. (2015) also performed photodegradation experiments for syn- and anti-DP dissolved in isooctane using a high-pressure mercury lamp (350-450 nm) at 20 °C for up to 80 minutes. No degradation was observed in dark controls. Degradation rate constants and half-lives were 0.014 and 50.96 min for the anti-isomer, and 0.013 and 53.72 min for the syn- isomer, respectively.

3.1.1.3.2 *Phototransformation in water*

i) A single non-guideline study was summarised in the registration dossier, assigned reliability 2 by the Registrant. Chou *et al.* (1979) measured the phototransformation of Dechlorane Plus in aqueous solution with 5 % acetonitrile using a mercury lamp emitting wavelengths >290 nm for 168 hours. This was compared with a similar solution kept in the dark as a control. The initial concentration was 1 ppm [1 mg/L], which is significantly greater than the reported solubility in pure water. Both solutions were extracted with methylene chloride and hexane, and the extract reduced in an evaporator before analysis using GC with electron capture. The evaporation step had an "uncertainty" of 10%, and this was reported as the "internal error" of the test system. One unidentified photolysis product was detected at less than 10% of the total concentration. The amount detected was within the internal error of the method of analysis, so the result can only be considered indicative. The rate constant for phototransformation in water was calculated as $<6 \times 10^{-4}$ h^{-1}, equivalent to a half-life of >48 days.

ii) An analogous substance, chlorendic acid, has been shown to be degradable in UV light (WHO, 1996), with a half-life of 5 days in water, 16 days in air, and 140 and 280 days in soil using two different concentrations. No other experimental details are provided, and the degradants are not specified. The reference in the IPCS assessment is to a proprietary study and so it is not possible to obtain further information[40]. The results suggest rapid degradation, but without further information, the results should be treated with caution.

Discussion

[40] The study is not included in the current REACH registration of the anhydride, and the acid is not currently registered (November 2014).

Although the study of Chou *et al*. (1979) suggests that photolytic degradation in water is a possibility, the findings cannot be related to the aquatic environment because the test solution included acetonitrile and significantly exceeded the reported solubility in pure water by several orders of magnitude.

3.1.2 Biodegradation

3.1.2.1 Biodegradation in water

3.1.2.1.1 *Estimated data*

The aerobic biodegradation potential of the substance can be assessed using BIOWIN v4.10 (U.S. EPA, 2012). It has six different models which have been developed based on expert judgment. The models employ fragment constants developed using regression analysis which are combined with substance molecular weight and defined model coefficients. The predictions for the non-linear model (BIOWIN 2), ultimate biodegradation (BIOWIN 3) and the MITI non-linear model (BIOWIN 6) can be used as a screening assessment of persistence (P) in accordance with the REACH Guidance R.7b (ECHA, 2017b). The following results indicate that a substance may be persistent:

BIOWIN 2: Does not biodegrade fast (<0.5) or

BIOWIN 3: ≥months (< 2.25 (to 2.75))

BIOWIN 6: Not readily biodegradable (<0.5) and

The predictions for the structure of Dechlorane Plus are BIOWIN 2: 0, BIOWIN 3: -1.60, and BIOWIN 6: 0. These values are all significantly below the cut-off values, indicating that Dechlorane Plus is not expected to be aerobically biodegradable.

These BIOWIN models are based on structural group contributions and there is no defined estimation domain as such. Parameters relating to the validity of each BIOWIN prediction are summarised below.

BIOWIN 2 uses a combination of fragments and molecular weight to make the prediction. The model is considered to correctly classify 93.2% of substances in the training set (n=295). The molecular weight of substances in the training set is between 31.06 to 697.7 so Dechlorane Plus (Mw = 654) is within the range. The training set contains 36 chemical fragments. The following model fragments are present in the Dechlorane Plus structure:

(a) aliphatic chloride x 12

(b) carbon with single bonds and no hydrogens x 6

There is no cycloaliphatic fragment, and so the octyl ring of Dechlorane Plus is not included in the estimate. Reviewing the specific substances in the training set also does not indicate any with the norbornene structure of Dechlorane Plus. Substances in the training set contain a maximum of 6 and 2 of fragments (a) and (b). This is lower than the 12 aliphatic chloride and 6 single bond carbon fragments in Dechlorane Plus meaning the prediction may be overly conservative. However, BIOWIN 2 still predicts a result of 0 if the number of fragments are set to the training set maximum (i.e. 6 and 2).

BIOWIN 3 also uses a combination of fragments and molecular weight to make the prediction. The model uses the same 36 chemical fragments and calculation method which is based on expert review of 200 substances. It is indicated to correctly

classify 83.5% of substances in the training set (n=200). The molecular weight range for the training set is 53.06 to 697.65 meaning Dechlorane Plus (Mw = 654) is within the range. BIOWIN 3 contains the same relevant model fragments for Dechlorane Plus as BIOWIN 2: aliphatic chloride and carbon with single bonds and no hydrogens. As with BIOWIN 2, the model prediction does not recognise the cycloaliphatic structure within Dechlorane Plus. The maximum number of aliphatic chloride fragments (3) and carbon fragments (3) in the BIOWIN 3 training set substances are also is lower than the number in the Dechlorane Plus structure. Applying a similar check as for BIOWIN 2, when the maximum number of fragments are used, BIOWIN 3 predicts a value of 0.598. A final aspect of the model applicability is that the Dechlorane Plus prediction value is outside of the regression plot of the training set substances, which could also affect the prediction reliability.

BIOWIN 6 uses a model fragment approach and is based on a training set of 884 substances. The model is considered to correctly classify 84.8% of the training set as not readily degradable and 82.3% of the validation set as not readily degradable. The larger training set contains 42 chemical fragments, and includes cyclic –CH2- and cyclic –CH- as well as the fragments used in BIOWIN 2 and BIOWIN 3. The number of each chemical fragment for Dechlorane Plus are within the maximum for the training set and the whole Dechlorane Plus molecule is accounted for within by the model fragments. The molecular weight Dechlorane Plus (Mw = 654) is also within the training set range of 46.07 to 885.46. The training set includes chlordane (CAS: 57-74-9) and heptachlor (CAS: 76-44-8) which contain the hexchloronorbornene moiety of Dechlorane Plus. BIOWIN 6 predicts a value of 0 for each of these substances which is validated by measured biodegradation data showing that they do 'not biodegrade fast'.

For all BIOWIN models, the model authors acknowledge that multiple occurrences of a contributing positive fragment group can sometimes lead to incorrect prediction of rapid degradation. In view of the group fragment values present in Dechlorane Plus, and the prediction of "not biodegradable", this is not considered to be an issue problem for the substance.

Overall the BIOWIN 2 and 3 model estimates have a degree of uncertainty due to i) the lack of fragment coefficients to represent the whole structure and, ii) the number of identified fragments exceeding the maximum of occurrence in training set substances. The latter issue does not appear to affect the prediction that Dechlorane Plus is not biodegradable. For BIOWIN 6, the Dechlorane Plus structure is fully represented by the model fragments. The number of each fragment in the Dechlorane Plus structure is also within the range of the training set. The training set substances include two chemicals, which contain the hexchloronorbornene moiety of Dechlorane Plus. The model predictions for those substances do agree with the measured data. Overall BIOWIN 6 is considered to be a relevant and reliable model for Dechlorane Plus. The model provides support to the BIOWIN 2 and 3 predictions as all three models are consistent in predicting that Dechlorane Plus does not biodegrade fast.

It is concluded that based on the estimated data associated uncertainties, that Dechlorane Plus is unlikely to be biodegradable.

3.1.2.1.2 *Screening tests*

Two old biodegradation screening studies were included in the registration dossier as key studies[41].

[41] A further study is mentioned under the heading "disregarded biotransformation and kinetics".

Boudreau (1973) measured the disappearance of the substance analytically over a period of 21 days. The inoculum used was a domestic activated sludge (non-adapted) (no information is available on the inoculum concentration). Initial nominal test substance concentrations of approximately 0.01, 1, 10 and 100 mg/L (active ingredient) were used. No positive or negative controls were included. Water was sampled on days 0, 7, 14 and 21. The stock solution of the substance was prepared in acetone, but precipitation was observed. Therefore only part of the nominal concentration could be detected analytically in the water samples. Initial analytical concentrations of 0.008 mg/L, 0.46 mg/L, 8.0 mg/L and 82 mg/L were determined by GC with electron capture. As the concentrations did not further decrease over the exposure time, this initial loss was not caused by biodegradation but by the non-homogenous distribution of the test substance in the stock solution. No biodegradation was observed over 21 days, as determined by analysis of the test substance. The Registrant assigned a reliability code of 2 (reliable with restrictions) to this study.

iii) Chou *et al.* (1979) performed a biodegradation study using radiolabelled Dechlorane Plus. The position of the radiolabel is not reported. The radiolabelled test item was incubated with sewage treatment plant sludge under aerobic or anaerobic conditions for 2 or 6 weeks at two nominal concentrations of 218 and 872 ng/mL [0.218 and 0.872 mg/L]. The total suspended matter concentration was 185 and 3 910 mg/L for the aerobic and anaerobic treatments, respectively. Parallel cultures were autoclaved to kill the sludge bacteria and served as negative controls. The water samples were extracted, and the radioactivity was determined by liquid scintillation. Degradation was determined as loss of radioactivity. No loss of radioactivity was seen after 2 weeks' incubation under aerobic or anaerobic conditions. After 6 weeks' incubation, the recovery of radioactivity under aerobic conditions was 42.9 % at the low concentration and 0.16 % at the high concentration when compared to 92.1 % in the sterile negative control at the high concentration. After 6 weeks' incubation under anaerobic conditions, the recovery of radioactivity was 87.5 % at the low concentration and 84.1 % at the high concentration, when compared to 88.4 % in the sterile negative control at the high concentration. It should be noted that different extraction methods were used at the 2- and 6-week analysis points. A mass balance, in terms of assessing radioactivity lost from the culture and extraction efficiencies, was not performed. Thin layer chromatographic (TLC) analysis of all fractions (from both anaerobic and aerobic experiments) at 2 and 6 weeks failed to identify the presence of any metabolites. The Registrant assigned a reliability code of 2 (reliable with restrictions) to this study. However, in their CSR, the Registrant states that the findings cannot be considered as proof for biodegradation because adsorption to sludge or bacteria cannot be excluded and the position of radiolabelling in the molecule is not described.

iv) The Registrant concluded that Dechlorane Plus is non-biodegradable.

Discussion

v) Neither of the two biodegradation studies reported by the Registrant was performed according to a standard guideline method. The study by Boudreau (1973) included no reference control and no blank control, determinations were not carried out in duplicate and there was no information on the concentration of

This was a company study performed in 1971, in which Dechlorane Plus was suspended in benzene and then added to test water at a nominal concentration of 4 ppm [mg/L]. The benzene was stripped by aeration, and then domestic sewage sludge was added, and the mixtures incubated for 30 days. No oxygen consumption was observed in either test or negative control cultures, but the sludge was viable after the 30 days' incubation. The Registrant assessed this study to be unreliable as an original report is unavailable (there is just a summary description in the form of a letter).

inoculum. In addition, both studies used chemical or radiometric analysis of the test substance to determine the extent of biodegradation. The standard OECD Test Guideline (TG) 301 for ready biodegradability advises that the methods that monitor biodegradation via dissolved organic carbon analysis (OECD TG 301A (DOC Die-Away) and 301E (Modified OECD Screening)) are not suitable for poorly soluble substances. Respirometry methods are recommended instead. The OECD TG 310 method (CO_2 in sealed vessels) is also recommended for poorly soluble substances. REACH Guidance R.7b (ECHA, 2017b) states that tests using DOC analysis cannot be used to assess the biodegradability of poorly water-soluble substances unless it is measured in addition to another parameter. This is because the substance might be removed from solution via adsorption to the inoculum or test vessel.

vi) Both studies significantly overdosed the test solution to a level well above the water solubility of the test substance, which should have resulted in most of the test substance being unavailable for biodegradation. In addition, the suspended substance could re-stock the solution to saturation concentration throughout the test, and thus any biodegradation taking place would not be observed by monitoring the test substance only. The losses observed by Chou *et al*. (1979) after six weeks of incubation could be the result of volatilisation or more likely adsorption of the substance to the test vessel or microbial inoculums – there was no mass balance, and TLC analysis of all fractions (from both anaerobic and aerobic experiments) failed to identify the presence of any metabolites by the end of the test.

In view of the inappropriate test methods used, and the additional deficiencies in the studies mentioned above, these biodegradation screening studies provide no reliable information on the biodegradation potential of the substance, and should not be considered valid. The Registrant draws a similar biodegradability conclusion, although considers the studies to be valid with restrictions.

It is also relevant to mention that a screening-level hazard characterisation by the U.S. EPA (2011) cites a Japanese biodegradation study where Dechlorane Plus achieved 0.6 % of its theoretical biochemical oxygen demand (BOD) in 2 weeks using an activated sludge inoculum and the modified MITI (OECD TG 301C) test. The reference stated by the U.S EPA for this biodegradation data is the NITE database "Biodegradation and Bioaccumulation of the Existing Chemical Substances" under the Chemical Substances Control Law. The test information on the English version of the J-Check website is a very limited summary (NITE, undated). The test substance concentration was 100 ppm and the sludge concentration 30 ppm. As well as the BOD measurement cited by U.S. EPA (2011), 0.3 % degradation was determined by gas chromatography. Correspondence with the Japanese Chemicals Evaluation and Research Institute (Yoshida, pers. comm., 2015[42]) confirmed that this study was actually conducted in 1974 and the report is simple without any raw data. There was no information about a reference substance, but it is likely to have been aniline. Cultivation was for 14 days only. This study is not mentioned in the REACH registration.

3.1.2.1.3 *Simulation tests*

3.1.2.1.4 *Field evidence*

de la Torre *et al*. (2011) sampled wastewater treatment plant (WWTP) sludge from 31 sites in Spain from April to June 2006. Statistically significant ($p < 0.05$) differences in Dechlorane Plus concentrations and isomer ratio were found in sludge

[42] Email from Tomohiko Yoshida to Ian Doyle, Environment Agency dated 29 January 2015.

from WWTP using biological nitrogen and phosphorous elimination compared to sites using biological digestion only. The data suggest that Dechlorane Plus might be susceptible to microbial degradation under some conditions, with the anti-isomer possibly more degradable than the syn-. However, there was significant overlap in concentration and isomer ratio in both types of WWTP, and the study provides no information on degradation rates.

3.1.2.2 Biodegradation in sediments

3.1.2.3 Biodegradation in soil

The Registrant has waived the biodegradation in soil simulation test based on considerations that the substance is highly insoluble in water, not hazardous and direct exposure to soil is unlikely.

Wang *et al*. (2010) reported the vertical distribution of Dechlorane Plus at different soil depths from sampling sites close to the Chinese manufacturing facility. The concentration was highest in surface soil (0-5 cm; 837 µg/kg dry weight (dw)), decreasing to 9.16 µg/kg dw at a depth of 60-70 cm and 3.84 µg/kg dw at a depth of 90-100 cm. The f_{anti} value varied little with soil depth, but ranged from 0.75 in the surficial layer to 0.67 in the deepest layer (90-100 cm). The study authors make conflicting statements in the paper about the implications. On the one hand, they said that this implied a stereoselective depletion of the syn- isomer in soil (in comparison with the measured f_{anti} value of the Chinese commercial product, at 0.60), but they also stated that isomer specific microbial degradation or preferential adsorption does not play a significant role in soil. This study is included in the registration dossier and is considered fully reliable by the Registrant.

vii) ***Discussion***

The data waiver is inappropriate. Although exposure considerations are a REACH Annex IX Column 2 adaptation for this endpoint, there is no definitive evidence that direct or indirect soil exposure is unlikely.

3.1.2.4 Analogue data

Measured data

The technical documents that supported the POPs agreements for each substance have been assessed for persistence information relevant to Dechlorane Plus. The data included in IPCS assessment (WHO, 1995) and supporting CICAD or Environmental Health Criteria (EHC) Monographs are summarised below. In some cases the physico-chemical data from these older assessments are different to the more recent values quoted in Section 1.5 of the main report.

1. Chlordane (CAS no. 57-74-9): Chlordane has a log K_{OW} of 6 and water solubility of 25 µg/L. The IPCS assessment includes one water-sediment degradation study conducted in a freshwater lake (WHO, 1984c). Although a half-life is not included in the assessment, the summary suggests that rapid dissipation to sediment occurs, followed by slow degradation. The mean concentration of chlordane in sediment was 35.29 µg/kg wet weight (ww) after 7 days and 10.31 µg/kg ww after 421 days (although a half-life is not stated). From the summary it is not clear whether the reduction in the sediment is dissipation or actual degradation.

   While residues in soil have been studied, none of the studies can easily be interpreted to provide a soil half-life. The POP overview document states that the half-life of chlordane in soil is approximately one year (UNEP, 1998).

While the source of these data is not referenced, this result would be in line with the sediment data. The chemical properties of chlordane – low water solubility, high stability and semi-volatility – are indicated to favour its long-range transport potential (LRTP) (WHO, 1995). The substance has been found in the air, water and organisms in the Arctic (WHO, 1995).

2. Heptachlor (CAS no. 76-44-8): Heptachlor has a log K_{OW} of 4.4 – 5.5 and water solubility of 180 µg/L. According to the IPCS assessment, the substance has a half-life of up to two years in soil (WHO, 1984a). This is based on a study performed in 1980, but there is little additional information provided in the assessment. A further study using pesticide application rates determined a half-life of 9-10 months.

   Another study cited in the IPCS report described the application of heptachlor to grass pasture: after 30 days, 4 % of the heptachlor remained, which reduced to 2 % when measured at 15 weeks. However, it is unclear whether this reflects degradation or some other loss process (such as volatilisation, or uptake onto/into the grass).

   Heptachlor is said to hydrolyse rapidly in water (forming 1-hydroxy chlordene), which is then microbially degraded to a further metabolite (WHO, 1984a). Formation of this hydrolysis product has also been observed in moist soil. This specific reaction is unlikely to be relevant to Dechlorane Plus as it does not contain the relevant unsaturated pentyl fragment. A further degradant, heptachlor epoxide has also been observed in soil, which appears to be a result of microbial degradation. The chemical properties of heptachlor – low water solubility, high stability and semi-volatility – are indicated to favour its LRTP (WHO, 1995). EFSA (2007) also highlights that the two break-down products heptachlor epoxide and photoheptachlor are persistent and lipophilic. Heptachlor and its epoxide have been found in air, water and organisms in the Arctic (WHO, 1995).

3. Aldrin (CAS no. 309-00-2) / Dieldrin (CAS no. 60-57-1): The water solubility is 17-180 µg/L for aldrin and 140 µg/L for dieldrin. The log K_{OW} of aldrin and dieldrin is 7.4 and 6.2, respectively. Aldrin is converted to dieldrin via epoxidation by micro-organisms and plants under aerobic conditions in soil (WHO, 1989). Transformation to the dicarboxylic acid is also described, although this appears far less significant. By contrast dieldrin is reported to be much less susceptible to biodegradation, with very little degradation detected in soil. Photodegradation has been studied for both chemicals but is not considered to be significant in the environment. The chemical properties of aldrin and dieldrin – low water solubility, high stability and semi-volatility – are indicated to favour their LRTP. Both aldrin and dieldrin have been found in air, water and organisms in the Arctic (WHO, 1995).

   The same conclusions also apply to endrin (CAS no. 72-20-8). Biodegradation of endrin occurs under anaerobic conditions, with the major metabolite being the ketone, delta-ketoendrin. Photodegradation can also occur, to form the same degradant, especially where endrin has been applied to crops (WHO, 1992 & 1995).

4. Endosulfan (CAS no. 115-29-7): Endosulfan has a water solubility of 0.33 mg/L and log K_{OW} of 4.78 - 4.93 (α- and β- isomers) (UNEP, 2009). The substance was considered to be photolytically stable (EFSA, unpubl. a). A soil simulation study performed using a range of soils under aerobic conditions determined DT_{50} values of between 12-39 days and 108-264 days for α- and β-Endosulfan respectively at 21-22°C (EFSA, unpubl. a). A further study conducted at a higher temperature of 28°C found half-lives of 23 and 58 days respectively (EFSA, unpubl. a). Longer half-lives would be expected at more environmentally relevant temperatures. The main degradant is

endosulfan sulfate, which is also biologically active, but more persistent with half-lives measured between 123 to 391 days (EFSA, unpubl. b). A number of water-sediment studies are available with rapid dissipation observed in water. The degradation and kinetics in sediment and the total system were complex and affected by pH, but a number of the metabolites were considered to have DT_{50} values above 120 days.

All of the metabolites in the degradation pathways observed in soil and water-sediment are a result of the degradation of sulfonate ring, and so the chlorinated norbornene ring remained intact. Little mineralisation (<5 %) was observed in any of the studies.

5. Chlorendic acid (CAS no. 115-28-6): Chlorendic acid has a very low log K_{OW} (-1.59). The water solubility is reported to be 499 mg/L. No mineralisation of the parent anhydride was observed by day 31 in a ready biodegradation study based on dissolved organic carbon removal. This confirms the lack of an obvious rapid degradation pathway for the chlorinated norbornene moiety. However, without information on primary degradation, it cannot be confirmed that the substance itself is stable from this test. There are no simulation data.

Based on the available information above, it appears that the chlorinated norbornene moiety is unlikely to undergo rapid degradation.

3.2 Environmental distribution

3.2.1 Adsorption/desorption

Assessment of adsorption/desorption has been waived by the Registrant based on the low solubility of Dechlorane Plus in water.

The Registrant also reported a study by Chou *et al.* (1979) in Section 5.4.4 (Other distribution data) of the IUCLID dossier. This was an investigation of adsorption to sediment, and was conducted by mixing a saturated water solution with water and about 10% of sediment overnight. Fractions (supernatant and bottom) were extracted separately using 15 % methylene chloride in hexane. The extracts were filtered and analysed using GC with electron capture. Concentrations between about 22 ng/L and about 44 ng/L were detected in the supernatant fraction, while the sediment (bottom) fraction contained between 74 and 136 µg/g (corresponding to about 74 and 136 mg/L) of Dechlorane Plus. A sorption partition coefficient of $4.5 \pm 1.9 \times 10^6$ was reported (the particulate matter was assessed from the blank control and considered for calculation). However, precipitation of Dechlorane Plus in the sediment phase was observed indicating that only a part of the substance concentration in sediment was adsorbed to the sediment.

viii) ***Discussion***

The data waiver for assessment of adsorption/desorption to be inappropriate. Insolubility in water is not a REACH Annex VIII Column 2 adaptation for this endpoint.

The study reported by the Registrant investigating adsorption to sediment (Chou *et al.*, 1979) is a non-standard study which is not similar to the OECD test guideline methods for adsorption/desorption, which determine a soil organic carbon-water partition coefficient (K_{OC}) at adsorption equilibrium. The study gives no useable information on adsorption behaviour, as the test method was very basic and precipitation occurred in the sediment phase.

3.2.2 Volatilisation

The transfer of a substance from the water phase to the gas phase can be estimated by means of the Henry's Law Constant (HLC). The HLC can be calculated from the ratio of the vapour pressure (VP) to the water solubility (WS).

HLC = (VP × MW) / WS

where MW is molecular weight (g/mol).

The vapour pressure value selected for the purposes of this assessment is 0.8 Pa 200 °C (see Section 1.5 of the main report). The corresponding vapour pressure value at 25 °C is 4.6×10^{-4} Pa, calculated using EUSES v2.1.2.

The water solubility value selected for the purposes of this assessment is $\leq2\times10^{-6}$ mg/L at 20 °C (see Section 1.5 of the main report). The corresponding water solubility value at 25 °C is $\leq2.16\times10^{-6}$ mg/L, calculated using EUSES v2.1.2.

An HLC of $\geq1.39\times10^{5}$ Pa.m^3/mol at 25 °C is calculated for Dechlorane Plus, based on these data.

The HLC can also be calculated using the 'structural fragment'-based QSAR method HENRYWIN v.3.20 (U.S. EPA, 2012), giving a value of 0.8 Pa.m^3/mol at 25°C. The Bond method training set comprises much smaller molecules than Dechlorane Plus, which are generally much more soluble and of higher vapour pressure than the substance, although the predicted HLC value is mid-range for the method. The number of C-C and C-Cl fragments in the substance exceeds the maximum occurrences of this fragment in a single compound in the training set. It is therefore difficult to estimate the uncertainty of the predicted value.

3.3 Potential for long range transport

Several studies report Dechlorane Plus levels in remote regions, including the Arctic and Antarctic. These are summarised in Table 6 for various environmental compartments (biota concentrations in remote regions are reported in Appendix 3 and 4). From the available information it appears that Dechlorane Plus can routinely be detected in air samples in remote regions at levels up to a few tens of pg/m^3, mainly associated with particulates, and that deposition to surface media occurs with these particulates.

Okonski *et al*. (2014) investigated the distribution of Dechlorane Plus on different size fractions of atmospheric particulates/aerosols in order to better understand the atmospheric transport potential of particle-bound Dechlorane Plus. According to Okonski *et al.* (2014), particles with an aerodynamic diameter <2 µm are removed less effectively from the atmosphere by wet or dry deposition than larger particles and so tend to remain in the atmosphere longer. For the study, samples of ambient outdoor air were collected at two sites in the Czech Republic over a one year period (October 2009 and October 2010). The sites were an urban site in central Brno and a rural site near the village of Telnice. A total of 46 samples were collected at each site, each representing approximately 1 week's duration, and the concentrations in various size fractions of the atmospheric particles (<0.49 µm, 0.49-0.95 µm, 0.95-1.5 µm, 1.5-3.0 µm, 3.0-7.2 µm and 7.2-10 µm) were determined. The target chemicals were detectable in approximately 72 % of the samples (including Dechlorane Plus; other chemicals were also included in the study but the detection frequency was given for the total chemicals rather than individual substances). Where the concentration was below the limit of detection (LoD) a value of LoD/2 was used for statistical purposes.

The concentration of Dechlorane Plus in the total atmospheric particulates (<10 µm) varied seasonally. For the urban site the levels of anti-DP atmospheric particulates ranged from 0.0696 pg/m^3 in summer to 0.232 pg/m^3 in autumn and the levels of syn-DP ranged from 0.503 pg/m^3 in summer to 0.279 pg/m^3 in spring. Similarly for the rural site the levels of anti-DP range from 0.0689 pg/m^3 in summer to 0.267 pg/m^3 in spring and the levels of syn-DP ranged from 0.113 pg/m^3 in summer to 0.366 pg/m^3 in spring.

At both sites it was found that 33.7 %±9.7 % (mean±standard deviation) was associated with particles <0.49 µm diameter and 57.4 %±7.9 % was associated with particles <0.95 µm/diameter. The mass median diameters of the particulates were on average 0.87 µm for the urban site and 0.78 µm for the rural site and did not vary significantly through the year. Both anti- and syn-DP showed variable concentrations across the size fractions investigated, with no strong peak in concentration on the finest fractions (as was found for some of the other substances studied) and there were seasonal differences in the size distribution. It was hypothesised that because Dechlorane Plus has a very low vapour pressure, it enters the atmosphere mainly via abrasion processes rather than volatilisation.

Okonski *et al.* (2014) also carried out modelling of wet and dry deposition potential for particle-bound Dechlorane Plus taking into account the different size-fractions of the particulates. This concluded that atmospheric deposition estimates considering different size fractions generally resulted in atmospheric deposition fluxes for Dechlorane Plus around a factor of 1.7 times lower than estimates obtained considering bulk atmospheric particulates only. This is a result of the high proportion of particles <0.49 µm diameter that may be transported longer distances and are less easily deposited or scavenged than larger particulate fractions.

Table 6: Abiotic monitoring data for Dechlorane Plus in remote locations

Compartment	Location	Findings	Reference
Atmosphere	Two remote research stations: one in Canadian high Arctic (Alert) and the other in the Tibetan Plateau (Nam Co)	Sampling dates not explicitly stated for Alert (but appear to be in the period 2006 to 2007). Monthly-integrated samples were collected between October 2006 and February 2008 at Nam Co. Detected in 11 out of 14 samples at Alert with total DP ranging from <0.05 pg/m^3 to 2.1 pg/m^3, primarily associated with particles. Not detected in pre-screening at Nam Co, which is suggested by the study authors to be due to fewer particulates reaching the station.	Xiao *et al*. (2012)
	Transects in East Greenland Sea, and northern and southern Atlantic Ocean	Sampling took place during August – September 2009 (Greenland) and November – December 2008 (Atlantic), 10 samples from each transect, with sampling over 2-6 days at 15 m a.s.l. DP was detected in all air samples, at concentrations of 0.05 – 4 pg/m^3. DP was mainly detected in the particulate phase. In the Atlantic, the highest concentration was observed in the English Channel originating from continental air passing Western Europe. The fractional abundance of syn-DP increased with decreasing northern latitude from 0.37 to ca. 0.67, showing a stereoselective depletion of anti-DP (thought to be caused by UV sunlight).	Möller *et al*. (2010)
	Transect from East China Sea to Arctic	17 air samples taken between June and September 2010. Each sample was collected over 1-2 days (height not specified). DP was detected in all samples in the range 0.01 – 1.4 pg/m^3 (as total DP), mainly in the particulate phase. Includes remote areas, but not exclusively.	Möller *et al*. (2011)
	Transect in Indian, Pacific and Southern Oceans from southeast Asia to Antarctica	Sampling took place during November 2010 – March 2011 (n=20). Each sample was collected over 1-2 days at 20 m a.s.l. DP was detected in all samples as follows: Pacific Ocean: 1.7-11 pg/m^3 (total DP) Indian Ocean: 0.26 – 2.1 pg/m^3 (total DP) Southern Ocean: 0.31 pg/m^3 (total DP)	Möller *et al*. (2012)
	Station Nord, Northeast Greenland	Detected in 46 % of air samples collected weekly throughout 2012. Syn-DP concentration: mean 2.32 pg/m^3 (range <1 - 9.0 pg/m^3). Anti-DP concentration: mean 5.24 pg/m^3 (range <1- 33.1 pg/m^3).	Vorkamp *et al*. (2015)

Compartment	Location	Findings	Reference
	Longyearbyen, Svalbard, Arctic (78.22°N 15. 65°E) Longyearbyen is a coal mining community with 2 100 residents and so a local source of Dechlorane Plus (e.g. building and pipe insulation) cannot be ruled out.	Samples were collected from September 2012 to May 2013 (a total of 34 samples, each collected over 48 hours). The samples represent the atmospheric particulate fraction collected on quartz fibre filters (2.2 µm cut-off). Total DP concentration was 0.05-5 pg/m^3. Anti-DP was detectable in 91 % of the samples with a mean (± standard error) of 1.1 (±0.19) pg/m^3. Syn-DP was detectable in 91 % of the samples with a mean (± standard error) of 0.29 (±0.04) pg/m^3. The f_{anti} ranged from 0.43 to 0.9 with a mean value of 0.75, which is reasonably consistent with that in commercial Dechlorane Plus products.	Salamova *et al*. (2014)
	Råö (Swedish west coast), Pallas (Northern Finland), Aspvreten (Swedish east coast, 70 km SW of Stockholm) [These represent 'background' sites.]	Samples were apparently collected in May, July and November 2009 and January 2010. DP concentrations were: Råö: 0.18 – 0.52 pg/m^3 (n=4) Pallas: 0.016 – 0.047 pg/m^3 (n=2; January and July only) Aspvreten: 0.12 - 0.23 pg/m^3 (n=3; January, July and November) Deposition fluxes were estimated from the monthly average data as follows: Råö: 0.11 – 0.34 ng/m^2/d Pallas: 0.017 – 0.025 ng/m^2/d Aspvreten: 0.15 - 0.39 ng/m^2/d	Kaj *et al*. (2010)
	Review of Arctic data	Does not cite articles beyond the ones already summarised here	Vorkamp *et al*. (2014)

Compartment	Location	Findings	Reference
Atmosphere	Northern Sweden	Bimonthly bulk atmospheric deposition samples were taken for one year from October 2009 to November 2010. DP was detected on all seven sampling occasions at Abisko in the Arctic (68°20'N, 9°03'E) at a maximum amount of 5.7 ng per sample (total isomers). DP was detected on three of five sampling occasions at Krycklan in the sub-Arctic (64°14'N, 19°46'E; located approximately 60 km northwest of the city of Umeå) at a maximum amount of 0.16 ng per sample (total isomers). The average (±standard deviation) of the monthly deposition flux for total DP was calculated to be 22±2.1 ng/m^2/month at Abisko and 1.1±0.52 ng/m^2/month at Krycklan. It is interesting to note that the higher flux was found at the more remote site. Newton et al. (2014) considered the air-parcel back trajectories at both sites. Both sites receive airflow from the west off the Norwegian and Barents Seas, and from the eastern and southern Baltic countries. However, Abisco is around 100 km from the Norwegian coast and receives a higher proportion of its air from the ocean than the continent, whereas a higher proportion of the air at Krycklan comes from the south and east and the air from the ocean passes over the land mass of Norway and Sweden. Thus the differences in fluxes at the two sites may reflect differences in emission sources. The fraction of the anti- isomer was lower at the Arctic site (mean 0.25) than the sub-Arctic site (mean 0.62), suggesting isomer-selective degradation or isomerization during long range transport to the more remote site. The f_{anti-} at the sub-Arctic site was similar to that in commercial products, and may indicate proximity to a local source.	Newton *et al*. (2014)
	Entebbe, Lake Victoria, Uganda	Weekly air sampling between October 2008 and July 2010. DP was not detected in 9 samples from 2008. In the 30 samples from 2009, syn-DP was detected in 17 % of samples (arithmetic mean: 0.21 pg/m^3) and anti-DP in 10% of samples (arithmetic mean: 0.10 pg/m^3). The geometric mean was below the detection limit for both isomers. In 17 samples from 2010, syn-DP was detected in 18 % of samples (arithmetic mean: 0.46 pg/m^3; geometric mean 0.05 pg/m^3) and anti-DP in 76 % of samples (arithmetic mean: 0.33 pg/m^3; geometric mean 0.18 pg/m^3). The paper notes that recycling of electronic equipment may be the source of the contamination.	Arinaitwe *et al*. (2014)
	All continents except Antarctica	Samples collected July – September 2005 (possibly also 2006) for a Global Atmospheric Passive Sampler (GAPS) study (25 sites, number of samples not stated). Reported concentrations ranged from 'not detected' to 348 pg/m^3, the highest level being for Cape Grim, Tasmania where population density is very low. It was also detected in north Alaska and Svalbard.	Sverko *et al*. (2010a) [ABST]

Compartment	Location	Findings	Reference
Seawater	Transects of sampling in East Greenland Sea, northern and southern Atlantic Ocean	Sampling took place during August – September 2009 (Greenland) and November – December 2008 (Atlantic). 10 samples from each transect. Concentrations in sea water were <detection limit – 1.3 pg/L, and DP was mainly detected in the particulate phase. Includes remote areas, but not exclusively.	Möller *et al*. (2010)
	Transect of sampling from East China Sea to Arctic	18 sea water samples taken between June 2010 and September 2010, with each sample collected over 12-24 h. Seawater concentrations ranged between 0.006 – 0.4 pg/L. Includes remote areas, but not exclusively.	Möller *et al*. (2011)
Seawater, sediment, soil and air	Ny-Ålesund, Svalbard (78°55'N, 11°56'E).	Samples of soil, moss and dung were collected simultaneously (see Appendix 4 for the moss and dung results). The mean (and range) of concentrations in the soil samples was 0.042 (0.012-0.105) µg/kg dw) for anti-DP and 0.284 (0.094-1.01) µg/kg dw for syn-DP. The f_{anti} in the soil samples was 0.18. Sediment and seawater samples were also taken at King's bay. The mean (and range) measured concentrations were 32 (85-648) pg/L and 61 (22-116) pg/L for anti-DP and syn-DP, respectively, in seawater; and 0.073 (0.023-0.228) µg/kg dw and 0.270 (0.085-0.648) µg/kg dw for anti-DP and syn-DP, respectively, in sediment. The f_{anti} was 0.36 in seawater and 0.21 in sediment. Na *et al.* (2015) considered that the low f_{anti} values found in water, sediment, soil (and also moss; see Appendix 4) may reflect degradation of anti-DP during long-range transport, possibly by UV.	Na *et al.* (2015)
Sediment	Kongsfjorden, Svalbard, Norwegian Arctic	27 samples of surficial sediment taken in July 2009. Syn-DP and anti-DP were detected in 78 % and 94 % of samples, respectively. Syn-DP ranged between n.d. - 5.4 pg/g dw (mean: 1.4 ± 1.5 pg/g dw). Anti-DP ranged between n.d. - 15.9 pg/g dw (mean: 4.5 ± 4.3 pg/g dw). No clear spatial trend between the outer and inner fjord. The paper notes that it is plausible that both glacial runoff and oceanic currents play a role in introducing DP to the fjord sediments. The relatively low fractional abundance of the syn-DP isomer indicates the long-range transport of this chemical to this Arctic site.	Ma *et al*. (2015)

3.4 Bioaccumulation

3.4.1 Bioaccumulation in aquatic organisms (pelagic and sediment organisms)

3.4.1.1 *Predicted data*

The bioconcentration factor (BCF) of an organic chemical can often be predicted using QSAR correlations with log K_{OW}, especially if the substance is not metabolised very readily. No predictions of bioaccumulation potential are made in the registration dossier. A measured log K_{OW} is not available in the registration dossier either, but it is estimated to be ≥9 in Section 1.5 of the main report. For the purposes of this assessment, some standard methods have been used to predict fish BCF values for Dechlorane Plus, although given the uncertainty in the actual log K_{OW} value they should be considered with caution.

ix) REACH Guidance R.7c (ECHA, 2014c) advises that the linear correlation relationship of BCF with log K_{OW} breaks down for substances with log K_{OW} values above 6. For these substances, bioaccumulation is limited by reduced bioavailability as a result of low aqueous solubility, slow membrane passage due to large molecular size, and by growth dilution, metabolism, degradation, etc. The equation recommended in the REACH Guidance R.7c is:

$$log\ BCF = -0.2 \times (log\ K_{OW})^2 + (2.74 \times log\ K_{OW}) - 4.72$$

For Dechlorane Plus, this equation gives a calculated BCF of ≤5 500 L/kg. However, the REACH Guidance indicates that whilst the model accounts for non-linearity above log K_{OW} values of 6, it is unreliable at log K_{OW} values above 8.

x) The BCFBAF v3.01 model (U.S. EPA, 2012) contains two main estimation methods. The first method estimates the BCF from the log K_{OW} by one of two regression equations (depending on K_{OW}), with corrections for specific substance classes.

For log K_{OW} >7.0: $log\ BCF = -0.49 \times log\ K_{OW} + 7.554 + \sum Correction\ factors$

This equation gives a calculated BCF value of ≤1 400 L/kg for Dechlorane Plus, using a log K_{OW} value of ≥9. No correction factors are used for this substance.

A review of the BCFBAF program has been carried out by the Environment Agency (EA) in the UK to determine the most relevant outputs from the model for use in the context of the EU REACH Regulation (EA, 2013). For substances with log K_{OW} >7 there is some uncertainty in the BCF database from which the BCFBAF regression based on log K_{OW} is constructed. This, and the fact that the BCFBAF regression for substances with log K_{OW} >7 was derived from a relatively limited number of data points, means that the BCFBAF log K_{OW}-based predictions for substances with log K_{OW} >7 are probably more uncertain than those for substances with log K_{OW} between 1 and 7. Therefore, there is uncertainty in the predicted BCF values for Dechlorane Plus using the log K_{OW} >7 regression equation.

xi) The second method in BCFBAF estimates BCF values and bioaccumulation factors (BAFs) for three trophic levels using a method based on a paper by Arnot and Gobas (2003).

EA (2013) indicates that there are many assumptions inherent in the Arnot-Gobas model potentially leading to uncertainty in the predicted BCF and BAF. It recommends that if the BCFBAF program is used, the BCF estimates from the regression equations are generally given preference over those from the Arnot-Gobas model for comparison with the REACH criteria. If the Arnot-Gobas predictions themselves are to be used, EA (2013) recommends that the preferred values for comparison with the REACH criteria should be the BCF values obtained for the lower trophic level. In the case of Dechlorane Plus, the BCF value obtained for the lower trophic level is 1 200 L/kg (using a log K_{OW} value of 9); the estimated bioaccumulation factor (BAF) value is 7.5×10^5 L/kg ww[43]. It is also relevant to note that the BCFBAF program estimates the BCF (and BAF) on the basis of the total concentration in water rather than the dissolved concentration in water (EA, 2013). The freely dissolved concentration of Dechlorane Plus would be significantly lower than the total concentration and hence the actual BCF based on dissolved concentrations may be higher than predicted above.

Chou *et al.* (1979) describe equations to calculate steady-state (maximum) rough estimates of BCF for organic compounds from known values of aqueous solubility, sediment-water partition coefficient and octanol-water partition coefficient. The calculated BCF values for Dechlorane Plus in this paper range from 5 200 to 1.6×10^7 (units not provided). However, the methods used are not recommended in the REACH guidance, and the equations based on log K_{OW} value lead to much higher BCF values compared to the other estimates provided above.

3.4.1.2 *Laboratory studies*

3.4.1.2.1 *Aqueous exposure*

The following BCF studies were reported in the registration dossier:

i) Chou *et al*. (1979) exposed twelve Bluegill Sunfish (*Lepomis machrochirus*) under static conditions to water containing 283 dpm[44]/mL (nominal) radiolabelled Dechlorane Plus. The test substance was solubilised in hexane prior to addition to water. No further details are reported on the radiolabelled test substance. Three fish were analysed at 48 hours and three more at 96 hours exposure. The remaining six fish were transferred after 96 hours of exposure to clean water, and were analysed at 48 hours and 96 hours post-exposure. The concentration of the substance in all fish and in water during period of exposure was determined by liquid scintillation counting. There is no information in the robust study summary to indicate whether the fish were given food during the uptake phase of the experiment, but given the relatively short study duration, presumably they were not.

   The BCF was determined as 7.02 and 1.97 (whole body, w/w) after 48 and 96 hours exposure, respectively. Measured concentrations of the test substance in fish were 1 798 dpm/mL after 48 hours and 614 dpm/mL after

[43] Sverko *et al*. (2011) included estimated biotransformation rate constants with this model to derive BAFs of 5.9×10^4 L/kg for the anti- isomer and 1.1×10^5 L/kg for the syn- isomer, based on total water concentrations. BAFs at the middle and upper trophic levels were higher.

[44] This acronym is not explained in the robust study summary, but presumably refers to "disintegrations per minute".

96 hours. Measured concentrations of test substance in the water were 256 dpm/mL after 48 hours and 311 dpm/mL after 96 hours.

After transferring the fish to clean water, high and highly variable concentrations were found in fish tissue at 48 and 96 hours post-exposure: 25 843 dpm/mL and 1 299 dpm/mL, respectively. No conclusion on the elimination from fish tissues could be drawn. The results of the depuration phase were reported as being caused by "oral intake of particulate precipitated test substance" by the fishes.

The Registrant assigned a reliability code of 2 (reliable with restrictions) to this study, on the basis that concentrations in fish were extremely variable (most probably by ingestion of particulate precipitated test substance), and concludes that there was no relevant bioconcentration during 96 hours of exposure in fish. A copy of the original study report is not available.

ii) In a second non-guideline study reported by the Registrant (Zitko, 1980), groups of three juvenile Atlantic Salmon (*Salmo salar*) were exposed under static conditions to a mixture of chemicals including Dechlorane Plus in water for 96 hours followed by 192 hours of observation in clean water. The test substance was a mixture of compounds in hexane containing a small amount of toluene (the identity of the other chemicals in the mixture was not specified in the robust study summary, but the original paper indicates that they were mirex, Dechlorane 602, Dechlorane 603 and Dechlorane 604). Following addition to the bottom of a flask the solvent was completely evaporated and then 3 L of water added (no information is provided about the time allowed for dissolution). The water contained 76.15 µg/L nominal (6.06 µg/L measured) Dechlorane Plus. The test temperature was 10 °C. The same study involved a dietary exposure group, and this part of the study is reported separately in Section 3.4.1.2.2.

Concentrations in fish tissue were determined after 12, 24, 48 and 96 hours of exposure, and 25.5, 102 and 192 hours post-exposure, using gas chromatography-mass spectrometry (GC-MS). Two peaks in an area ratio of 1:4 were believed to be the syn- and anti- isomer, respectively, and concentrations were reported as total isomers.

Dechlorane Plus was not detected in fish tissue during the exposure or depuration periods, i.e. there was no measurable uptake from water after 96 hours' exposure. (In contrast, mirex and Dechlorane 602 were both accumulated.)

The Registrant assigned a reliability code of 2 (reliable with restrictions) to this study, on the basis that methods, results and the interpretation of results are not described in sufficient detail.

iii) Gara and Rawisina (1975) exposed one group of 36 Bluegill Sunfish (Latin name not provided, presumably *Lepomis macrochirus*) to a nominal concentration of 0.1 ppm [mg/L] commercial Dechlorane Plus for 30 days in a flow-through test system. There was no depuration period. Dechlorane Plus was used as a 0.001 % solution in acetone. This solution was injected at 90-second intervals beneath the water surface of the test tank. The study did not follow a standard guideline. Samples of fish were taken pre-test and on days 0, 6, 12, 18, 24 and 30. Samples of water were taken pre-test and on days 0, 6, 18, 24 and 30. Analysis was performed by gas chromatography. No further details on the analytical methods are available.

Measured concentrations in water were 0.102 ppm at day 0 (6 hours); 0.095 ppm at day 6; 0.074 ppm at day 12; 0.083 ppm at day 18; 0.085 ppm at day 24; and 0.069 ppm at day 30. The concentration in fish increased

from 0.111 ppm on day 0 to 0.385 ppm on day 30. The BCF was calculated as 5.58 (using the concentrations measured at the end of the study).

No effects on mortality and behaviour were observed that could be attributed to the substance. Quiescence, dark discoloration of the integument, rapid respiration and passive feeding were considered to be due to acetone (which reached 0.7 % v/v).

The Registrant has assigned the study Reliability 2 and indicates that steady state was not achieved during 30 days of exposure.

iv) Boudreau and Rausina (1973) performed a 30-day static bioaccumulation study with juvenile Bluegill Sunfish (Latin name not provided, presumably *Lepomis macrochirus*). The fish were exposed to a nominal concentration of 1 ppm [mg/L] Dechlorane Plus (dissolved in acetone) in a static system for 30 days. The test substance was introduced once on day 0, the water was aerated, but the oxygen concentration was not measured. No information on water quality is reported. There was no depuration period. Samples of water and fish were collected pre-test and on days 7, 14, 21, and 30, and analysed by gas chromatography after extraction with cyclohexane.

Measured concentrations in water were 0.046 ppm at day 7; 0.014 ppm at day 14; 0.001 ppm at day 21; and 0.003 ppm at day 30. Measured concentrations in fish were <0.01 ppm at day 0; 6.03 ppm at day 7; 7.80 ppm at day 14; 8.78 ppm at day 21; and 8.72 ppm at day 30 (suggesting a BCF of 2 907 – 8 780 L/kg during the last week or so of the study).

No effects on mortality and behaviour were observed, although an algal bloom occurred in all vessels in the second week.

The study has been determined to be non-reliable (Reliability 3) by the Registrant because the test substance purity was not reported, steady state was not achieved, the measured concentrations were significantly lower than nominal concentrations and there was likely to be precipitation of the substance and subsequent ingestion by the fish.

The four studies cited above are also cited in Occidental Chemical Company (2003) (which does not contain any other bioaccumulation data).

The following information was not included in the registration dossier by the Registrant, but has been identified following a literature search (refer to the Annex for information on search terms/period):

v) A screening-level hazard characterisation of Dechlorane Plus conducted by the U.S. EPA (2011) reports that Dechlorane Plus has measured BCF values for Common Carp (*Cyprinus carpio*) in the range 23 – 121 L/kg at 0.0027 mg/L and 14 – 96 L/kg at 0.00027 mg/L. The source of these data is the Japanese NITE database "Biodegradation and Bioaccumulation of the Existing Chemical Substances under the Chemical Substances Control Law". This information no longer seems to be available on the Japanese CHemical Risk Information Platform (CHRIP) database[45]. The data were reviewed under a Cefic Long Range Research Initiative project[46], and the data are summarised in the BCF gold standard database that resulted[47] as well as the

[45] http://www.safe.nite.go.jp/english/db.html (checked July 2014).

[46] http://www.cefic-lri.org/lri-toolbox/bcf

[47] http://ambit.sourceforge.net/euras/

OECD QSAR Toolbox (v2.1)[48]. These provide the following details. The study was carried out by Japanese regulatory authorities (MITI, referencing "Chemicals Inspection and Testing Institute (1992)"). It involved both male and female fish, but fish numbers, age and weights are not specified. The fish were apparently 8 cm in length (presumably at the start of the test; growth rate information is not provided). The test was conducted under flow-through conditions at 25 °C and pH 6.0 - 8.5. Total organic carbon (TOC) measurements are not provided. The uptake phase lasted 56 days, and there was no depuration period. Fish whole body was analysed, and the fish lipid content was reported as 4.8 % (it is not stated what time point this represents). The steady state BCF was based on the mean measured water concentration "up to the time of sampling", although no further information is provided. The wet weight steady state BCF is reported in this source to be 96 L/kg (at 0.27 µg/L) and 121 L/kg (at 2.7 µg/L). The Cefic project considered the study to be reliable, although the basis for this is not explained.

Correspondence with the Japanese Chemicals Evaluation and Research Institute (Yoshida, pers. comm., 2015[49]) confirmed that this study was actually conducted in 1974, and that the report is simple and does not contain raw data. The test substance was dissolved in methanol and 53 mg/L methanol solution was used as a stock solution, but the concentration of methanol in the test water was not stated. No information is available on lipid content or growth, but the average weight and length of fish during the test period were approximately 37 g and 11 cm, respectively. The following table provides the results from the study report (there is no information of the concentration measurements in fish).

		Value at week				
		2	**3**	**4**	**6**	**8**
Exposure Level 1	Concentration in test water, µg/L [Nominal: 2.65] (n=1)	2.71	2.54	2.21	2.04	1.87
	BCF (n=2)	*26*	*33*	*83*	*84*	*121*
		23	*32*	*59*	*99*	*98*
Exposure Level 2	Concentration in test water, µg/L [Nominal: 0.27] (n=1)	0.41	0.37	0.34	0.35	0.31
	BCF (n=2, but n=1 after 2 weeks)	*14*	*40*	*41*	*50*	*96*
		-	*32*	*34*	*48*	*87*

The decline in aqueous test concentration (and increase in BCF values) during the study indicates that the test system was not at steady state.

Discussion

None of the studies provides any information about differences in bioaccumulation potential of the two geometric isomers.

The solubility of Dechlorane Plus in pure water is significantly below 1 µg/L. Although there are uncertainties in measuring a precise value, from the discussion in Section 1.5 of the main report, the combined solubility of both geometric isomers is likely to be below 2 ng/L (0.002 µg/L) at 20 °C. A measured log K_{OW} is not

[48] http://www.oecd.org/chemicalsafety/risk-assessment/theoecdqsartoolbox.htm

[49] Email from Tomohiko Yoshida to Ian Doyle, Environment Agency dated 29 January 2015.

available in the registration dossier, but the substance is clearly very hydrophobic and for the purposes of this report, it is estimated to be ≥9. These properties make aqueous studies very difficult to perform, because the substance will have a high tendency to adsorb to organic matter and glassware (ECHA R.7b, 2008), limiting availability to fish and making it difficult to maintain test concentrations. The time for a strongly hydrophobic substance to reach equilibrium in an organism may also be longer than the standard exposure time for regulatory bioconcentration tests (Hawker and Connell, 1986).

Both 'key' studies in the registration dossier are non-guideline aquatic exposure studies. The actual exposure concentration in the Chou *et al*. (1979) study is unknown, as it is only expressed in terms of radioactive count. The number of fish (12) and exposure duration (4 days) are much lower than recommended by the current standard guideline (OECD TG 305-I, Aqueous Exposure Bioconcentration Fish Test). For example, modern tests would typically expose the fish for 28 days, and there was no evidence that a steady-state concentration in fish had been reached in this study[50]. Indeed, the concentration in fish was highly variable over the four sampling times (including depuration). The study authors believed this was due to fish ingesting different amounts of the particulate precipitated test substance following transfer to clean water. It is not clear what the source of this precipitate might be. Additional shortcomings include the use of static rather than flow-through or semi-static conditions, lack of a control group, and no information reported on fish lipid content. Therefore, the BCF values obtained in this study cannot be considered to be reliable, and in the view of the DS the study is invalid.

The same problems affect the Zitko (1980) study, which also included other substances that might have affected the uptake or metabolism of Dechlorane Plus, as well as toxicity towards the test fish. The fact that the measured aqueous concentration was more than ten times lower than nominal suggests that a significant amount of substance was not in solution. Even the measured value (6.06 µg/L) exceeds the solubility in pure water by a factor of around 3 000, so it is unlikely to provide a realistic indication of the actual dissolved, bioavailable amount of substance that the fish were exposed to. A BCF cannot be estimated since a limit of detection (LoD) for the analytical method for fish tissue is not available. In view of the short exposure time and very low number of fish, this study is invalid and does not provide any relevant information for the bioaccumulation assessment.

Two further bioaccumulation studies included by the Registrant exposed the fish for a longer period of 30 days. The Gara and Rawisina (1975) study was a flow-through study and therefore potentially more suitable for a hydrophobic substance, although it did not follow a standard test guideline. The method of test substance addition (a solution in acetone was injected at 90-second intervals beneath the water surface of the test tank) is not in accordance with current guideline methods, which recommend continuous dispensing and dilution of a stock solution of the test substance. The measured test concentrations (0.069 - 0.102 ppm) were over ten thousand times higher than the likely solubility limit of Dechlorane Plus in pure water, and it is likely that precipitation of the test material, adherence onto the

[50] Annex V of the standard OECD TG 305 states that an estimate of the depuration rate k_2 (day^{-1}) may be obtained by the following empirical relationship:

$$log\ k_2 = 1.47 - 0.414\ log\ K_{OW}$$

For Dechlorane Plus, k_2 is estimated to be to 0.0055 day^{-1} using a log K_{OW} value of 9. An estimate of the time to steady-state cannot be made as this would require information on fish weight, which is not available from either BCF study reported by the Registrant. However, the calculated rates of depuration (k_2), and corresponding half-life of 126 days, indicate that it is likely to take many days for steady-state concentrations of Dechlorane Plus in fish to be reached.

food given and subsequent ingestion of the test material took place. The measured aqueous concentration also fell to 69 % of the nominal exposure concentration by day 30, so the exposure regime might not have been constant. There is no information on the lipid content of the fish, and no depuration was carried out. The calculated BCF of 5.58 is based on the ratio of measured concentrations in fish and in water on day 30. A kinetic BCF cannot be calculated as there was no depuration period. Steady-state bioconcentration should be calculated based on the ratio of concentration in fish and in the water at steady-state. The standard OECD TG 305 (Bioconcentration in fish: aquatic and dietary exposure) states that a steady-state is reached in the plot of test substance in fish (C_f) against time when the curve becomes parallel to the time axis and three successive analyses of C_f made on samples taken at intervals of at least two days are within ±20% of each other, and there is no significant increase of C_f in time between the first a last successive analysis. The three final fish concentration measurements were 2.15 ppm (18 days), 0.320 ppm (24 days) and 0.385 ppm (30 days). Therefore, it cannot be concluded that steady-state had been reached. Based on these shortcomings, in the view of the DS the Gara and Rawisina (1975) study cannot be considered to be reliable. If it were assumed that the bioavailable fraction equates to the likely solubility limit pure water of (less than) 0.002 µg/L, the tissue concentration at day 30 (0.385 mg/kg) would suggest a non-steady state BCF above 100 000 L/kg. However, the likely oral ingestion of the substance as a precipitate and/or adsorbed to food would mean that there was more than one uptake route (as indicated in Section 3.4.1.2.2, dietary uptake is not insignificant), so this value is not a reliable indication of bioconcentration potential.

The Boudreau and Rausina (1973) study involved static exposure and so is not reliable, as demonstrated by the decline in aqueous concentrations over the period from a nominal concentration of 1 ppm at day 0 to 0.003 ppm at day 30 (apparently influenced by an algal bloom). Even the final measured concentration is over a thousand times higher than the likely solubility limit in pure water. It is also possible that water quality parameters such as pH and oxygen could have deteriorated to levels comprising fish health at all treatment levels before the end of the study. Concentrations in fish also increased over the exposure period, and so it cannot be determined whether steady state was reached. This study is therefore invalid, and the reliability ranking of the Registrant is appropriate. Assuming that the bioavailable fraction equates to the water solubility limit of (less than) 0.002 µg/L, the tissue concentration at day 30 (8.72 mg/kg) would suggest a BCF above 10^6 L/kg. However, since it is likely that there was more than one uptake route, this value is not a reliable indication of bioconcentration potential.

The Japanese BCF study cited by U.S. EPA (2011) is unreliable as steady state was not achieved (reliability 3). It was a standard guideline study performed under flow-through conditions, but is forty years old. Many older Japanese studies used high levels of dispersant and non-specific analytical techniques, and relevant information is not available for this study. Two test concentrations were used: the higher one (2.7 µg/L) exceeded the likely solubility limit in pure water by a factor of over 1 000. The lower concentration (0.27 µg/L) is over a hundred times higher. Back-calculating the wet weight fish concentration from the reported maximum BCFs (0.327 and 0.0259 mg/kg at the two exposure concentrations, respectively), and then assuming that the bioavailable fraction equates to the solubility limit in pure water of 0.002 µg/L allows a BCF of well over 10 000 L/kg to be estimated. As with the other studies discussed above, this estimate might not be reliable if the fish were also exposed to the substance adsorbed to food or particulates.

In summary, despite the validity marking given by the Registrant, none of the BCF studies reported in the registration dossier provide reliable data on the bioaccumulation behaviour of Dechlorane Plus. The longer duration studies do indicate that the substance can be taken up into fish tissues. If it is assumed that

the fish were exposed to the substance at its water solubility limit, tentative BCFs are estimated to be well above 10 000 L/kg, although the likely oral exposure of the fish means that these values might be a misleading indication of bioaccumulation potential. The maximum fish concentrations measured in the aqueous BCF studies were 0.385 – 8.72 mg/kg after 30 days for *Lepomis macrochirus* (Gara and Rawisina (1975) and Boudreau and Rausina (1973)) and 0.327 mg/kg after 56 days for *Cyprinus carpio* (Japanese BCF study cited by U.S. EPA, 2011). The wide range for *L. macrochirus* could be due to experimental variation (especially as the exposure conditions were not robust) or could perhaps be linked to differences in lipid content and age of the test fish. It is not known if these measurements would have included any substance adsorbed to the skin or present in the gut. If steady state had not been reached it is possible that the concentrations could become higher with longer exposures.

Other species

As well as the above information on bioconcentration in fish, a recent study by Zhao *et al.* (2014) has investigated the bioconcentration of Dechlorane Plus in the Sea Lettuce (*Ulva pertusa*), a macroscopic seaweed. The study was carried out using a 21-day exposure period followed by a 14-day depuration period. The mean measured Dechlorane Plus concentration used in the test was 1 351±101 ng/L and the test solution was prepared by dilution of a stock solution of Dechlorane Plus in acetone (the amount of acetone in the test solution was 0.01 % v/v). A solvent control was also run. It should be noted that the concentration in water exceeds the water solubility of Dechlorane Plus by around a factor of 1 000 (see below).

The organisms used in the study were obtained from the intertidal zone of the Yellow Sea near Dalian China. Pieces of the organisms (initial mean wet weight 0.46 g) were held in 100 mL of sterilized f/2 medium (pH 8.2) and cultivated at 10 °C. Following acclimation for at least two weeks, 21 pieces of the organism were individually exposed to the Dechlorane Plus solution and 18 pieces were individual exposed to the control solution for 21 days. The organism were then placed in clean test solution and allowed to depurate for 14 days. The concentration of Dechlorane Plus in the organism was determined in days 0, 7, 14 and 21 of the uptake phase and days 0, 7 and 14 of the depuration period (three replicates at each sampling time).

The mean concentration of syn-DP and anti-DP in the organisms at the start of the test were 4.38 and 14.13 µg/kg ww. The concentration of anti-DP and syn-DP were found to increase during the exposure period and the highest concentrations were reached after 14 days' exposure (34.17 µg/kg ww for syn-DP and 62.03 µg/kg ww for anti-DP). The concentrations in the control organisms were in the range 2.56-6.92 µg/kg ww for syn-DP and 10.93-20.21 µg/kg ww for anti-DP during the experiment.

Zhao *et al*. (2014) calculated the uptake and depuration rate constants for syn-DP to be 0.164 day^{-1} and 0.337 day^{-1} respectively. Assuming that the BCF is the ratio of these two rate constants, the kinetic BCF can be estimated to be 0.49. Similarly the uptake and depuration rate constants reported by Zhao *et al.* (2014) for anti-DP were 0.083 day^{-1} and 0.236 day^{-1}, giving a kinetic BCF of 0.35. However, these rate constant values may not have been estimated correctly (see below). In addition, based on the measured concentrations in the organisms at day 14 of uptake (the total Dechlorane Plus concentration would be 34.17+62.03 = 96.2 µg/kg ww) and the initial concentration of Dechlorane Plus in water (1.35 µg/L; see below) this would suggest a steady state BCF of the order of at least 71.

There are a number of uncertainties with this study that mean the results should be treated with caution. Some of these are outlined below.

- The specific growth rate (SCR in units of % per day) of the organism was stated to be calculated according to the following equation: $SCR = 100 \times (W_{t2} - W_{t1})/t$, where W_{t2} is the weight at time 2, W_{t1} is the weight at time 1 and t is the time interval between time 2 and time 1. However, this equation does not give the percentage growth per day but rather the mass increase per day.

- Inhibition of the growth of the organism compared to the control appears to have occurred on day 7 and 14 of the uptake phase. The specific growth rates for these two time points are given as -2.36 % per day and -0.97 % per day. This suggests that the Dechlorane Plus may have exerted a toxic effects during the uptake phase. The paper indicates that the specific growth rate returned to similar levels as the control during depuration. As noted above the equation used to estimate the specific growth rate can be questioned.

- The paper incorrectly assumes that growth dilution can be ignored since the effects of Dechlorane Plus on growth inhibition were limited. Growth inhibition and growth dilution are not the same. From the specific growth rates given it appears that the organisms may have actually been decreasing in weight during some parts of the study.

- Dechlorane Plus was present in the organisms at the start of the test, and was detectable in the control organisms throughout the test.

- The initial concentration in water was 1.35 µg/L, which exceeds the water solubility of Dechlorane Plus. The concentration in water declined during the uptake period and was <300 ng/L by day 7 of the uptake and was in the range 50-70 ng/L during depuration. Thus the exposure was not constant during the uptake phase and uptake could still have been occurring during the depuration phase.

- The kinetics were fitted using a two box model that included volatility loss, the metabolic rate constant and the depuration rate constant. However the study only determined the concentrations in water and the concentrations in the organism, and it is not possible to distinguish between all of these processes using these data. In particular there are several aspects that may have been oversimplified in the analysis. Firstly, the authors assumed that the loss of Dechlorane Plus from the solution without organisms was due to volatilisation loss, but there may be other explanations for this, e.g. adsorption to vessel walls, etc. (the initial concentration in water (1.35 µg/L) exceeded the water solubility of the substance). Secondly, the authors neglected the depuration processes that would have been occurring when estimating the uptake kinetics (the concentrations in the organisms at any one time during the exposure part of the experiment represent the net uptake (i.e. uptake from water and depuration from the organism)). The uptake model used in the paper effectively assumes that the decline in the water concentration is a result of uptake into the organisms and loss through volatilisation and uses the decline in water concentration to indirectly estimate the rate constant for uptake into the organism. Finally, the rate constant for metabolism appears to have been estimated by omitting other depuration processes from the model.

3.4.1.2.2 *Dietary exposure*

In addition to Tomy *et al*. (2008), the registration dossier includes the following dietary exposure studies:

a) In a non-guideline study, Zitko (1980) fed groups of three juvenile Atlantic Salmon (*Salmo salar*) with food pellets coated with a mixture of chemicals dissolved in hexane that included Dechlorane Plus for 42 days followed by an observation phase of 71 days. The food contained 9.12 µg/g nominal (8.88 µg/g measured) Dechlorane Plus. The other chemicals in the mixture were not specified in the robust study summary, but the original paper indicates that they were mirex, Dechlorane 602, Dechlorane 603 and Dechlorane 604. The test temperature was 10 °C.

Concentrations in fish tissue were determined by GC-MS at 15, 28 and 42 days of exposure, and 16, 32, 49 and 71 days post-exposure. The total number of fish used in the study was not stated, but each sampling point included two fish analysed individually. Two peaks in an area ratio of 1:4 were believed to be the syn- and anti- isomer, respectively. This ratio did not change during the test, so concentrations were reported as total isomers. A maximum concentration of 176 ng/g ww Dechlorane Plus was detected in fish tissue at the first time point of analysis (15 days). The concentration decreased steadily thereafter, to 61.8 ng/g ww at 28 days and 44.2 ng/g ww by the end of exposure (42 d). The study authors noted that this may be an artefact as the fish had doubled in size (from 9.4 to 20.0 g) by uptake day 42. Lipid weights dropped during the test (from 6.45 % at day 15 to 3.66 % on day 42 of uptake, then 2.93 % on day 16 of depuration rising to 4.98 % by the end of the test), and it was thought that this could have been due to reduced ingestion and/or toxicity (fish weight had also dropped to 8.3 g by day 16 of depuration, rising to 19.7 g by the end of the test). Tissue concentrations decreased further during the depuration period to 18.7 ng/g ww after 71 days of depuration. Complete elimination was not achieved, and the non-growth corrected depuration half-life was 58 days (the depuration rate constant was 0.012 d^{-1}). A BMF was not reported, but it was concluded that all the test chemicals were accumulated when administered in food; the accumulation factor of mirex was said to be relatively high, and accumulation factors of Dechloranes "low to intermediate".

In the supporting information for a paper by Arnot & Quinn (2015), a growth rate constant of 0.0055 d^{-1} is estimated based on a relatively poor fit (r^2 = 0.32) of an exponential model to the reported fish mass data. The resulting growth-corrected depuration rate constant is 0.0065 d^{-1}, corresponding to a growth-corrected depuration half-life of about 100 days for ca. 15 g, 5 % lipid content fish.

The Registrant assigned a reliability code of 2 (reliable with restrictions) to this study, on the basis that methods, results and the interpretation of results are not described in sufficient detail.

b) Xiao *et al*. (2013) investigated the gastro-intestinal absorption efficiency of Dechlorane Plus as part of a study that exposed Rainbow Trout (scientific name not given but presumably *Oncorhynchus mykiss*) to fifteen chemicals simultaneously via the diet. Following acclimation to laboratory conditions for a month, six juvenile (13-month old, 25 g) fish were allocated to each of four 50-L stainless steel aquaria. After one day, the fish were fed with a single meal of 1.5 g contaminated feed (prepared by shaking a slurry of the chemical mixture dissolved in toluene with 2 mm food pellets for 16 hours, followed by drying for 20 hours). It is not stated whether all the fish were exposed to the same chemical mixture, but presumably this was the case. Five control fish were fed the same food but without the test chemicals. The water flow rate was 12 L/h, giving a water exchange rate of six times per day. The water temperature was maintained at 13 °C. All of the food was consumed within 10 minutes, and faeces were removed from the aquaria by siphoning twice a day (the slurry of faeces and water was filtered through

up to eight pre-weighed glass-fibre filters (depending on the amount of faeces); the filters were allowed to dry overnight and then kept in the dark at room temperature until extraction). After five days[51], the fish were killed and stored at minus 18 °C until analysis. Dechlorane Plus was analysed by GC-MS with electron capture negative ionization (ECNI). Procedural blanks were run in parallel for each batch of extractions. The feed was analyzed in triplicate, faeces samples were analyzed once, and fish samples were analyzed in duplicate.

The measured dose of Dechlorane Plus in the food was 1.9 µg (although not reported, the concentration would have been 1 270 µg/kg). The amount detected in the fish and faeces was 0.3 µg and 0.61 µg, respectively, giving an analytical recovery of 51 %. As the fish were unlikely to have grown significantly during the study, the approximate whole body concentration would have been 12 µg/kg, although this is not reported in the paper. The apparent absorption efficiency (calculated as the ratio of the amount of chemical in the fish to the sum of the amount in fish and faeces) was 0.37 (standard deviation 0.17; no other information about concentration variability is provided). Control fish were reported to contain negligible amounts of the test chemicals relative to the fish fed contaminated feed (the actual amounts are not stated).

Given the relatively poor analytical recovery (presumably due to either metabolism of the parent substance, degradation during the analytical procedure, poor extraction efficiency and/or measurement error), the accuracy of the absorption efficiency estimate is unclear. In addition, the gross absorption efficiency for a short-period, single-meal exposure is different from the net absorption efficiency for continuous dietary exposure (the calculation method is not the same as that required in OECD TG 305). The influence of co-exposure with the other substances on uptake kinetics is also unknown. The study is therefore unreliable for regulatory purposes, although it does imply that gastro-intestinal absorption following a single oral dose is not insignificant – a single exposure to 1 270 µg/kg in food led to a whole body concentration of around 12 µg/kg; when the amount in fish is compared to the amount in the food, at least 16 % of the dose appears to have been absorbed.

This study is not included in the registration dossier.

c) Zeng *et al*. (2014a) exposed 36 Common Carp (*Cyprinus carpio*) (approximately 12 cm in length) to commercial Dechlorane Plus via their diet to investigate gastrointestinal absorption and tissue-specific bioaccumulation in muscle, serum, liver and gonad (including unlaid eggs). The fish were kept in one tank at 22ºC, and four further fish were used as controls.

Samples were analysed using GC-MS. The anti- isomer was detected in the procedural blanks, and both isomers were detected in the control fish and non-spiked food, but at levels several orders of magnitude below those of the exposure group (e.g. average concentrations in the control fish were 34.7 and 19.7 µg/kg ww for the anti- and syn- isomers, respectively).

Contaminated feed was prepared by dissolving the substance in iso-octane followed by dilution in cod liver oil. 1 mL of this mixture was added to 10 g of fish food pellets, followed by homogenization in a shaking incubator for

[51] The time required for the fish to digest and pass a single meal was established to be less than five days in pre-experiments, and so the study duration was expected to minimize elimination via respiration, depuration from the fish body to the gastro-intestinal tract and biotransformation.

24 hours at 25 ºC. Fish were fed contaminated food for 50 days at a rate of 1 per cent of their body weight per day, followed by a 40-day depuration period using non-spiked food. Uneaten food was collected on days 5 and 45 of the uptake period and on days 55 and 90 of the depuration period. The average concentration of the anti- and syn- isomers in the spiked food was 7.8 and 2.1 mg/kg dw, respectively (anti- and syn-DP-1Cl were also present at 0.03 and 0.004 mg/kg dw, respectively). The dose of Dechlorane Plus was approximately 1.5 mg per day for each fish, and there was no fish growth during the study.

Faeces were collected on days 5, 10, 15, 25, 35, 40 and 50 (uptake period), and on days 60, 70, 80, 85 and 90 (depuration period). The Dechlorane Plus concentration in faeces during the uptake phase was in the range 1.5-5 times higher than in the spiked food, indicating a low absorption efficiency and/or a high excretion efficiency. The relative proportion of the anti- isomer in food was marginally higher than in faeces at each sampling point during uptake (except day 50). The study authors suggested that this indicates a slightly higher absorption efficiency for this isomer. The concentration in faeces at the first sampling point during depuration was around one order of magnitude lower than during uptake, with an exponential decrease thereafter. The faeces:non-spiked feed concentration ratio indicated that there were no systematic stereoselective differences in excretion for the two isomers.

Two fish were sampled every 5 days, dissected and the blood, liver and gonad tissues pooled for that day, resulting 36 fish carcasses, 18 serum, 18 liver and 18 gonad samples. Dechlorane Plus isomers were detected in all samples, although there was significant variation between individual fish. Normalization to total lipid content produced higher variations in fish tissue concentrations, so the wet weight data were used for the analysis.

The mean assimilation efficiencies of the anti- and syn- isomers were estimated to be 3.2 and 3.8 per cent, respectively, although the calculation method is not consistent with the recommendation of OECD TG 305-III. The study authors speculated that the lower assimilation efficiency of the anti-isomer could be attributed to stereoselective metabolism (although this difference may just be an artefact of the study and other explanations are possible).

Concentrations in the liver were higher than those in the muscle, serum and gonad throughout the experiment. Lipid content did not explain this difference (the liver lipid content (1.7 per cent) was significantly lower than that in muscle (3.4 per cent) and gonad (3.7 per cent). The liver also exhibited a high affinity for anti- isomers during the experiment. Other tissues, such as serum, muscle, and gonad, showed a selective accumulation of the syn- isomer in the early stages of the experiment, particularly the serum. A dynamic tissue distribution was observed, with an increasing proportion in the muscle along with a decreasing proportion in the liver over time. Indeed, due to its volume, muscle was the main tissue for deposition, accounting for more than 60 percent of the Dechlorane Plus accumulated during the entire experimental period, reaching 95 per cent by day 75 (though declining thereafter).

Uptake kinetics were linear in serum and muscle, with the highest concentrations recorded on day 50 (~280 and ~68 µg/kg ww for the anti-isomer, and ~80 and ~20 µg/kg ww for the syn- isomer, respectively (values read from a graph)). A steady-state was not reached in these tissues. In contrast, the uptake kinetics in liver and gonad were not linear. The highest concentration in liver was also measured on day 50 (~490 and ~125 µg/kg

ww for the anti- and syn- isomer, respectively (values read from a graph)), indicating that steady state had not been reached. However, the concentration in gonads peaked on day 40, at ~450 and ~125 µg/kg ww for the anti- and syn- isomer, respectively (values read from a graph).

Depuration in both muscle and serum was rapid for the first 10 days, followed by fluctuating concentrations over the remainder of the experiment with no obvious trend during the last 30 days of depuration (the concentrations on day 75/80 were in fact higher than on day 50). Depuration in the liver showed two-stage elimination kinetics, with an initial decrease to day 70, followed by a slight increase until the end of the experiment. A zigzag pattern was observed for gonads, with higher concentrations found than during uptake on some days.

The concentration ratios of dechlorinated analogues to their parent isomer in muscle decreased during the depuration period, suggesting that dechlorination is not a significant process in carp.

Overall, the study suggest that the bioaccumulation of Dechlorane Plus is a complex and multi-factorial process. This study is included in the registration dossier and is considered fully reliable by the Registrant.

d) Hang et al. (2013) [ABST] reported accumulation of concentrations of up to 1.9 mg/kg in adult Zebrafish (*Danio rerio*) following dietary exposure for 7 days (this study is summarised in Section 5.1.1.1.2 as it focussed on toxicity). Only limited details are available, and it is not clear whether the measurement excluded gut contents. Since actual food concentrations are not provided, it is not possible to estimate a BAF (some of the doses were above 1 g/kg bw/d, which may not be environmentally relevant). Its overall reliability cannot be assessed. These results therefore cannot be used for bioaccumulation assessment, but the data are provided for comparison with the other studies.

Discussion

Contrary to the validity marking given by the Registrant, the non-guideline study by Zitko (1980) with *S. salar* is not valid. The feed preparation technique did not conform to OECD TG 305-III requirements, and there are no details about the feeding regime (amount, frequency, etc.), number of fish or controls. The significant changes in fish size and lipid content during the uptake and depuration phases make the results very difficult to analyse. The study included other related substances that might have affected the uptake or metabolism of Dechlorane Plus, as well as the health of the fish. The study was also carried out under static conditions, whereas OECD TG 305-III recommends using either flow-through or semi-static conditions, to limit potential exposure to the test substance via water as a result of any desorption from spiked food or faeces. No BMF was calculated from the dietary exposure data, and in view of the uncertainties in the exposure regime and changes in the fish, it is not relevant to do so.

3.4.1.3 *Field studies*

Several biota monitoring studies have been included in the REACH registration dossier and identified by a literature search (refer to the Annex (page 190) of this report for information on search terms/period):

1. Wu *et al*. (2010) investigated the trophic transfer of Dechlorane Plus in a freshwater food web from a highly contaminated site in South China. A total of eighty-eight wild aquatic biota samples, six water samples, and six surficial sediment samples were concurrently collected from a reservoir near the e-waste recycling plants, South China (23.6021 N,

113.0785 E) in 2006. The biota samples comprised two invertebrates (Chinese mystery snail *Cipangopalundina chinensis* (n=43, 3 composite samples) and prawn *Macrobrachium nipponense* (n=7, 3 composite samples)), four fish species (Mud Carp *Cirrhinus molitorella* (n=12, 8 composite samples), Crucian Carp[52] *Carassius auratus* (n=18, 7 composite samples), and Northern Snakehead *Ophicephalus argus* (n=6)) and one reptile (water snake *Enhydris chinensis* (n=2)). Mud Carp (n=5) were also collected from another pond 5 km away from the e-waste recycling plant and used as reference samples. Stable isotope analysis of nitrogen was said to have been previously determined by the authors in another study. Trophic level was calculated using the equation $TL_{consumer} = [(\delta^{15}N_{consumer} - \delta^{15}N_{primary\ consumer})]/3.4 + 2$ (where 3.4 is the isotopic trophic enrichment factor).

Whole organism samples were extracted and Dechlorane Plus isomers were measured using gas chromatography/electron capture negative ionization (ECNI) mass spectrometry. Quality control procedures included recoveries of surrogate standards (CDE 99 and ^{13}C-BDE 209, 72.2 ± 19.8 % and 76.3 ± 6.9 %, respectively). Five spiking blanks with the Dechlorane Plus isomers and five procedural blanks were performed. The recoveries of the syn- isomer were between 101 % and 117 % with a mean of 107 %. The recoveries of the anti- isomer ranged from 92 % to 108 % with a mean of 102 %. No Dechlorane Plus isomers were detected in any procedural blanks. The limit of quantification was 4.4 and 1.9 pg/g for the syn- and anti- isomers, respectively.

The total Dechlorane Plus concentrations in the collected aquatic species ranged from 19 – 9 630 ng/g lw. The highest average concentration was found in water snake (1 970 ng/g lw), followed by Mud Carp (1 710 ng/g lw), Crucian Carp (277 ng/g lw), Northern Snakehead (255 ng/g lw), prawn (190 ng/g lw) and Chinese mystery snail (20.2 ng/g lw). The paper does not report wet weight data separately[53]. The average concentration on Dechlorane Plus in the water dissolved phase, suspended particles and surficial sediments were 0.80 ng/L, 3 930 ng/g dw and 7 590 ng/g dw, respectively. Dechlorane Plus was detected in all Mud Carp samples from the e-waste recycling site, but was detected in only one of the five reference fish, at a concentration of 8.76 ng/g lw.

With the exception of two Chinese mystery snail samples in which the syn- isomer was below the limit of quantitation, all of the biota samples had a higher syn-/anti- isomer ratio compared to sediment. Enrichment was highest in Northern Snakehead, which occupies the highest trophic level in the sampled food web. Bioaccumulation factors (BAFs) for Dechlorane Plus were calculated by dividing the concentrations of Dechlorane Plus in biota (ng/g wwt) by the mean concentrations of Dechlorane Plus in the dissolved phase of water (ng/mL). The average log BAF of total Dechlorane Plus ranged from 2.13 (Chinese mystery snail) at the base of the food web to 4.40 (water snake) near the top. All species except Chinese mystery snail and Northern Snakehead showed

[52] Common Carp *Cyprinus carpio* were also sampled but, unlike the other species, data are only provided in graphs. From the supporting information, it appears that only one individual of this species was involved, so its representivity is unknown.

[53] Lipid contents were water snake: 1.06 ± 0.15%, Mud Carp: 2.87 ± 0.41% (or 5.22 ± 0.78 % for the reference site), Crucian Carp: 3.63 ± 0.71%, Northern Snakehead: 1.49 ± 0.31%, prawn: 2.39 ± 0.32% and Chinese mystery snail: 0.59 ± 0.11%.

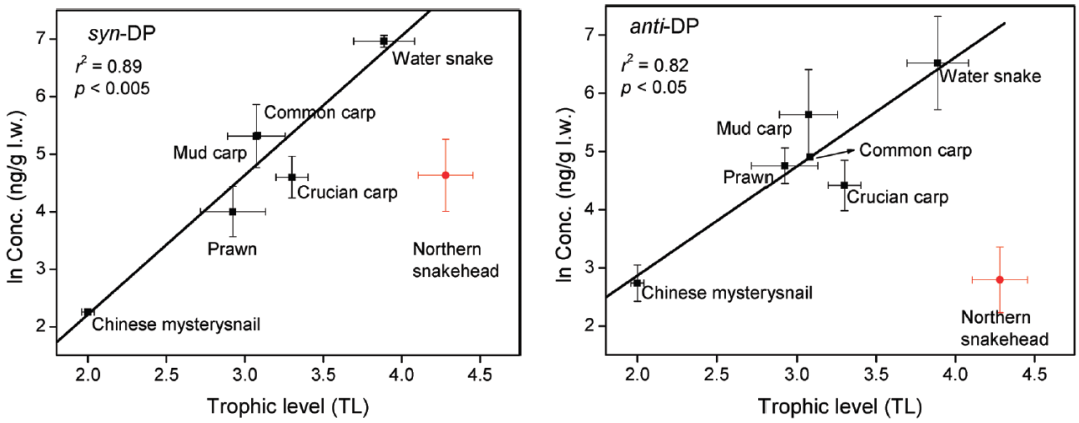
log BAF values higher than 3.70 (equivalent to a BAF of 5,000 L/kg[54]). The log BAF values of the syn- isomer were significantly greater than those of the anti- isomer in each species (paired samples *t* test, $p < 0.05$). This supports the hypothesis that fish may have a higher assimilation efficiency for the syn- isomer than for the anti- isomer. The highest wet weight concentration based on these data appears to be ca. 20 ng/g (µg/kg) wwt for the water snake.

The food web biomagnification potential of Dechlorane Plus was evaluated via estimation of trophic magnification factors (TMFs), which are derived mathematically from the slope of the regression model obtained from a plot of lipid-normalised contaminant concentrations in organisms versus trophic level. The plots in the paper are replicated in Figure 1.

Northern Snakehead had unexpectedly low concentrations considering its trophic position at the top of the food web, which significantly distorted the general relationship for Dechlorane Plus (the authors thought that this species might have been able to metabolise the substance more effectively than the others, and/or have a lower uptake rate for the anti- isomer). These data were therefore excluded when calculating the TMF. The calculated TMFs of the syn- and anti- isomers were 11.3 and 6.5, respectively (10.2 for total isomers).

Figure 5: Regressions between trophic level and concentrations of syn- and anti- isomers in aquatic species at a contaminated site in South China (bars represent ± 1 standard error)

(reprinted with permission from Wu *et al*. (2010). Copyright 2010: American Chemical Society)



The figure indicates that there was significant overlap in measured anti- isomer concentrations between some of the species, whereas there was clearer separation for the syn- isomer. Dechlorane Plus concentrations in biota measured in the study were 1 – 2 orders of magnitude lower than concentrations of BDE-47 and PCB-153 (the most abundant congeners found in the species studied) found in previous studies by the authors. However, the TMF for total Dechlorane Plus was approximately five times

[54] For total isomers, the average BAF was 12 200, 7 170 and 2 000 for Mud Carp, Crucian Carp and Northern Snakehead, respectively (Zhang *et al*., 2011c).

higher than that of total PBDEs (2.3), and was similar to that of total PCBs (11.1).

No information is provided in the paper about the age of the fish and reptile species sampled. Body burden might be linked with age, with older animals potentially accumulating more substance than younger ones due to increased exposure time (although growth dilution may limit the overall concentration). However, one of the study authors (Luo, pers. comm., 2015[55]) confirmed that although the age of the sampled species was not established, fish length (5-12 cm) suggests that the fish at least were not more than two years old. Zhang *et al*. (2011c) (see below) specifically said that the body length of the sampled Northern Snakehead (14.1 – 16.7 cm) indicated that they were 1–2 years old.

The paper does not indicate whether all the species sampled were in the same food web. The water snake feeds mainly on fish and occasionally amphibians[56], but it is not stated whether the size and species of fish included in this study would be part of its normal diet. In response, Luo (pers. comm., 2015) indicated that all the species sampled were from the same food chain as they were collected from the same small lake (100 m wide by 500 m long), and that the fish sampled would have been food items for the water snake[57].

It should be noted that the Northern Snakehead data indicate a lack of biomagnification (and even biodilution for the anti- isomer) in at least some feeding relationships. Only two snakes were sampled, so the representivity of the results are uncertain.

The three benthic-feeding carp species all have lipid normalised concentrations that are higher than both prawn and Chinese mystery snail, which seem likely to be part of their diet to some extent. However, these fish species are bottom feeders so may also be exposed to the substance adsorbed to sediment (which is significantly contaminated at this location – see below).

In addition, some other studies have suggested that factors other than lipid may be important for the bioaccumulation of this substance, which may make lipid normalisation of the data less appropriate.

Therefore, it appears reasonable to conclude that the study indicates biomagnification for some feeding relationships in this aquatic food chain, although given the small sample numbers involved, this conclusion is not unequivocal. This study is included in the registration dossier and is considered fully reliable by the Registrant.

Zhang *et al*. (2011c) reported biota-sediment accumulation factors (BSAFs) using the same data set. This study is also included in the registration dossier and is considered fully reliable by the Registrant. Average BSAFs for total isomers (defined as the lipid normalized concentration in the fish divided by the organic carbon normalized sediment concentration) were 0.004, 0.025, and 0.003 in Crucian Carp (n=6), Mud Carp (n=7) and Northern Snakehead (n=6) respectively.

[55] Email from Dr Xiao-Jun Luo to Ian Doyle, Environment Agency dated 27 January 2015.

[56] http://www.biosch.hku.hk/ecology/hkreptiles/snake/Enhydris_chinensis.html. It is possible that terrestrial species might also be eaten.

[57] Although uptake from air cannot be excluded for the water snake, dietary exposure seems likely to be more significant given the low vapour pressure of the substance.

These values are based on mean sediment concentrations from three samples. There is no information about how the BSAF varies with sediment concentration, but the study authors noted that much higher BSAFs were obtained in the studies of Shen *et al*. (2011) and Jia *et al*. (2011) (see below). They were derived on different bases, but the paper comments that the lower BSAFs in this study may reflect non-equilibrium conditions between sediment and fish (although there is no direct evidence for this).

Several extremely hydrophobic polychlorobiphenyls (PCB-199, -203, -207 and -208) and decabromodiphenyl ether (BDE-209) were also investigated in the same sample set. The average BSAFs for PCBs were higher than those for Dechlorane Plus, which was thought to reflect their higher bioavailability/biomagnification potential. However, the sediment concentrations of both Dechlorane Plus and BDE-209 were much higher than the PCBs, so if the levels in the organisms had reached saturation, the BSAF based on these high sediment concentrations could underestimate the level of bioaccumulation. Levels of BDE-209 in two of the fish species were in fact greater than all the other substances (levels of Dechlorane Plus were also higher than those of PCB-207 and -208 in all three species).

2. Tomy *et al*. (2007) assessed the extent of bioaccumulation of Dechlorane Plus using archived samples from Lake Ontario and Lake Winnipeg (Canada). Samples were said to have been collected between 2000 and 2003 (although the source documents suggest 2000 and 2002). Additional sediment samples from the central basin of Lake Ontario collected in 1998 were also used.

   The sampling regime is reported in two separate papers. For Lake Winnipeg, Law *et al*. (2006) reported that the biota samples were collected between 2000 and 2002 offshore of the town of Gimli on the south basin of the lake. Skinless muscle tissue samples were prepared for each fish, and mussels (zooplankton and some smaller fish species were processed as composites due to the limited quantity of sample material). Surface sediment (top 2 cm) grab samples (n = 4) were collected from four sites in the south basin of the lake. Biota samples included Walleye *Stizostedion vitreum* (n = 5), Burbot *Lota* (n = 5), Emerald Shiner *Notropis atherinoides* (n = 5), Whitefish *Coregonus clupeaformis* (n = 5), White Sucker *Catostomus commersoni* (n = 5), Goldeye *Hiodon alosoides* (n = 5), zooplankton (n = 5), and mussels *Lampsilis radiata* (n = 5).

   For Lake Ontario, Tomy *et al*. (2004b) reported that biota samples were collected between June and September 2002 at offshore stations. Sediment (n=3) was also sampled, but the locations are not stated. Biota samples included adult Lake Trout *Salvelinus namaycush* (whole fish, n = 4), Alewife *Alosa pseudoharengus* (composites of 5 fish, n = 4), Rainbow Smelt *Osmerus mordax* (composites of 5 or 20 fish, n = 4), Slimy Sculpin *Cottus cognatus* (composites of 10 or 15 fish, n = 4), mysids *Mysis relicta* (composites of >100 individuals, n = 1), amphipods *Diporeia hoyi* (composites of >100 individuals, n = 3) and zooplankton (n=1). The invertebrates and forage fish (Alewife, Smelt and Sculpin) were processed as composites of whole individuals, whereas all Lake Trout (a top predator fish species) were individual whole fish.

   Biological samples were homogenized with dry ice, spiked with a suite of recovery internal standards, and extracted using accelerated solvent extraction, lipid removal by gel permeation chromatography, and further

clean-up using Florisil. Samples from the two lakes were analysed at separate laboratories, using identical methodologies involving GC-mass selective detectors. Quality control procedures included the use of chlorinated diphenyl ethers and mass labelled hexabromocyclododecane isomers as recovery internal standards. There was no statistically significant difference between the recoveries of the recovery standards or Dechlorane Plus isomers, and no recovery correction was applied to the data. Duplicates of Sculpin from Lake Ontario, extracted separately and analyzed to check for repeatability, were within 94 % of each other for both isomers suggesting good repeatability. An interlaboratory comparison using four sediment extracts gave good agreement (63 to 86 %) between the measured Dechlorane Plus isomer values. Trace amounts of both isomers (syn-, 0.6 pg; anti-, 2.6 pg) were present in the blanks. Using an average sample mass of 15 g, method detection limits were 0.3 and 1.5 pg/g (µg/kg) for syn- and anti- isomers, respectively. Fish wet weight concentrations are not reported in the paper (nor its data supplement).

In Lake Winnipeg, the syn- isomer was consistently detected in all samples, while the anti- isomer was less frequently detected (ca. 45 % of samples). Lipid-corrected and blank-adjusted concentrations of the syn- isomer were greatest in Burbot (median: 415 pg/g lipid, range: 67 – 773 pg/g lipid), zooplankton (median: 542 pg/g lipid, range: 469 – 647 pg/g lipid) and mussels (median: 504 pg/g lipid, range: 76 - 823 pg/g lipid). Concentrations of the anti- isomer were greatest in the higher trophic level species Walleye (median: 714 pg/g lipid, range: 608 – 883 pg/g lipid) and Goldeye (median: 763 pg/g lipid, range: 594 – 932 pg/g lipid). Concentrations of both isomers were similar in Whitefish but varied considerably in Walleye and Goldeye; respective concentrations of the anti- isomer were 25 and 14 times greater than that of the syn- isomer in these species. Concentrations in sediment were small (11.7 pg/g dwt syn- isomer and 18.3 pg/g dwt anti- isomer). Total Dechlorane Plus concentrations were 2-3 orders of magnitude lower than those of total PBDEs and total hexabromocyclododecanes in biota from Lake Winnipeg. (In sediments, total Dechlorane Plus concentrations were only slightly less than total hexabromocyclododecane concentrations, suggesting that the former substance has lower bioavailability.)

In Lake Ontario (which is downstream of a manufacturing site), both isomers of Dechlorane Plus were detected in all samples, with the anti- isomer at consistently higher concentrations than the syn- isomer. Similar median concentrations of both isomers were observed in Lake Trout (syn = 44.3, anti = 47.2 pg/g lipid), Smelt (syn = 5.5, anti = 6.5 pg/g lipid), Alewife (syn = 48.3, anti = 54.2 pg/g lipid) and Sculpin (syn = 626, anti = 777 pg/g lipid). Concentrations of both isomers were greatest in the lower trophic level benthic organism *Diporeia* (syn, 1 307 ± 554; and anti, 3 108 ± 898 pg/g lw) and also high in zooplankton (syn, 719; and anti, 1 332 pg/g lw). In addition, concentrations of anti-Dechlorane Plus were approximately 2.5 times greater than syn-Dechlorane Plus in *Diporeia*, three times greater in *Mysis* and two times greater in plankton. Concentration of the anti- isomer in sediment was ~85 % of the total Dechlorane Plus concentration (mean 206 ng/g dwt), which were orders of magnitude greater than sediment concentrations in Lake Winnipeg.

In Lake Winnipeg, trophic level-adjusted BMFs (based on the ratio of lipid-corrected concentrations) were calculated for Walleye/Whitefish, Walleye/Whitesucker, Walleye/Goldeye, and Goldeye/zooplankton

feeding relationships. These were all below 1 for both syn- and anti- isomers, with the exception of the Walleye/Whitefish predator-prey relationship (BMF of 11, for the anti- isomer only). In Lake Ontario, trophic level-adjusted BMFs were calculated for Lake Trout/Alewife, Lake Trout/Smelt, Lake Trout/Sculpin, and Sculpin/*Diporeia* feeding relationships. The only BMF >1 was for the Lake Trout/Smelt predator-prey relationship, for both isomers (12 for the syn- isomer and 11 for the anti- isomer). BMFs of 1.0 (syn-) and 0.9 (anti-) were also obtained for the Trout/Alewife feeding relationship.

These results suggest that interspecies differences in bioaccumulation and biotransformation are likely. However, the BMFs assume that a predator consumes one prey species only, and as this is unlikely to be the case in reality, they should be treated with caution.

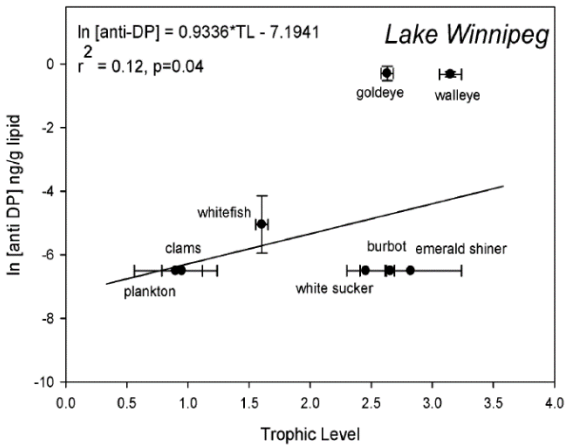
Regressions of the trophic level against concentrations were statistically significant for both isomers only in the Lake Winnipeg food web. The trophic positions of the species sampled in Lake Winnipeg, based on ^{15}N:^{14}N ratios and assuming a constant enrichment factor, were as follows: mussel → zooplankton, Whitefish → Goldeye, White Sucker → Burbot, Walleye (*Stizostedion vitreum*) (top predators). The lipid weight TMF value was 2.5 (r^2 = 0.12, p = 0.04) for the anti- isomer (see Figure 2), suggesting biomagnification, and 0.45 (r^2 = 0.17, p = 0.01) for the syn- isomer, suggesting biodilution.

It can be seen that concentrations of species other than Goldeye and Walleye were similar, which explains the poor correlation.

For Lake Ontario, plots using both wet weight (not presented in the paper) and lipid weight concentrations failed to give a statistically significant relationship for either isomer. The study does not discuss whether all the species sampled in a particular lake were in the same food web. However, based on information from the Great Lakes Environmental Research Laboratory[58], it seems that they were (the species in the Lake Erie food web documented in the sources are similar to the species sampled in Lake Winnipeg). Nevertheless, the analysis places Emerald Shiner at a higher trophic level than Whitefish in Lake Winnipeg, which is unexpected based on their ecology and feeding habits. The Emerald Shiner is a relatively small fish (up to ~13 cm) that feeds mainly on microcrustaceans, midge larvae and algae, whereas the Whitefish is larger (up to ~100 cm) and similarly feeds on aquatic insects, molluscs and amphipods, but also other fish and fish eggs (information taken from www.fishbase.org).

[58] http://www.glerl.noaa.gov/pubs/brochures/foodweb/LEfoodweb.pdf, and http://www.glerl.noaa.gov/pubs/brochures/foodweb/LOfoodweb.pdf.

Figure 6: Regression between trophic level and concentrations of the anti- isomer in aquatic species in Lake Winnipeg (error bars represent ± 1 standard error)



(reprinted with permission from Tomy *et al*. (2007). Copyright 2007: American Chemical Society)

The results from Lake Ontario were based on whole fish samples, whereas those for Lake Winnipeg were based on fish muscle only. These might not reflect whole body concentrations, and so this introduces some uncertainty in any comparisons and the analysis. Levels in the biota samples were also relatively low (generally < 1 µg/kg lw), and the very small sample sizes and possibility of variable sediment exposure (as samples were not necessarily collected from the same location or at the same time) raise additional uncertainties about the representivity of the data for these food chains. Other studies have suggested that factors other than lipid may be important in the accumulation of this substance, so lipid normalisation might not be relevant.

This study is included in the registration dossier and is considered reliable with restrictions by the Registrant. Overall, this study indicates that uptake of Dechlorane Plus occurs in wild fish, either directly from the dissolved phase or via food. However, the results and reported conclusions must be interpreted with caution: whilst the study suggests that interspecies differences in bioaccumulation/ biotransformation are likely, and that biomagnification may be taking place in some aquatic food webs and feeding relationships, the evidence is equivocal.

3. Mo *et al*. (2013) investigated Dechlorane Plus levels in Common Kingfishers (*Alcedo atthis*) and their prey fish collected at the same time from an electronic waste (e-waste) recycling site and a reference site in southern China. Total concentrations on a lipid weight basis in whole fish were similar to or higher than those in the birds (pectoral muscle) collected at the same locations (see Appendix 3**APPENDIX 3** for the actual values), suggesting that trophic magnification was not occurring in this food chain. This assumes that muscle is a representative tissue for the birds. This study is not included in the registration dossier.

4. Klosterhaus *et al*. (2012) conducted a survey of flame retardants in San Francisco Bay, USA. Surface sediments (top 5 cm) were collected in 2007 from ten spatially distributed sites. Shiner Surfperch (*Cymatogaster aggregata*) (n=8 composite samples, 20 fish per composite) and White Croaker (*Genyonemus lineatus*) (n=6 composite samples, five fish per composite) were collected in 2006 from popular recreational fishing sites

in three areas. These two species were selected because they are known to contain high concentrations of organic chemical contaminants. Three composites of Double-crested Cormorant (*Phalacrocorax auritus*) eggs (seven eggs per composite) were collected in 2008 from active nests at a single site. Harbour Seal (*Phoca vitulina*) blubber was sampled from fresh dead or freshly euthanized stranded seals from locations in the Central Bay segment in 2007 and 2008 (four adult females, one adult male, six female pups and six male pups). Following sample work-up, extracts were analyzed using gas chromatography and mass spectrometry operated in electron capture negative ionization mode (GC/ECNI-MS). Quality control measures included the analysis of blanks, duplicates, matrix spikes and standard reference materials. Data were blank corrected by subtracting the average mass in the blank samples; detection limits for these samples represent three times the standard deviation of the laboratory blanks.

Dechlorane Plus was detected in all of the sediment samples at a median concentration (total isomers) of 0.2 ng/g dw (range: 0.1 – 0.9 ng/g dw), which is similar to the levels of hexabromocyclododecane (HBCDD) that were found (median: 0.3 ng/g dw). Dechlorane Plus was detected in most of the wildlife samples, although at levels close to the detection limit. Median concentrations (total isomers) were 0.5 ng/g lipid weight (lw) (range: <1.0 – 1.8 ng/g lw) in White Croaker (detected in 83 % of samples, anti- isomer only), 1.3 ng/g lw in Shiner Surfperch (range: <8.0 – 3.7 ng/g lw, detected in 75 % of samples, anti- isomer only), 0.9 ng/g lw in Double-crested Cormorant eggs (range: 0.9 – 1.1 ng/g lw, detected in 100% of samples, syn- isomer only), and <0.1 and 0.9 ng/g lw in Harbour Seal blubber from pups and adults, respectively (range: <0.1 - 0.1 ng/g lw for pups and 0.2 – 7.1 ng/g lw for adults, detected in 8 % and 42 % of pup samples (anti- and syn- isomer, respectively) but 100% of adult samples (both isomers)). For comparison, levels of HBCDD were 6.0, 6.5, 37.4, 3.5 and 7.1 ng/g lw, respectively; levels of PBDEs were one to two orders of magnitude higher. The fractional abundance of the syn- isomer could only be determined for adult seal (mean 0.54, range: 0.51 – 0.57) and sediment samples (mean 0.35, range: 0.2 – 0.6), and the difference suggests that the syn- isomer may be more bioaccumulative than the anti- isomer.

An analysis of trophic magnification was not performed. Whilst the data imply that whilst Dechlorane Plus is present throughout the food web, there is no obvious trend with trophic position. In addition, the comparative bioaccumulation potential of PBDEs and HBCDD is higher.

This study is included in the registration dossier as supporting monitoring information and is considered fully reliable by the Registrant. Although the bioaccumulation aspects are not discussed in the CSR, the study summary does mention the isomer distribution difference between seals and sediments.

5. Peng *et al.* (2012) investigated tissue distribution, maternal transfer and age-related accumulation of Dechlorane Plus (and other substances) in wild-caught female Chinese Sturgeon collected between 2003 and 2006 at the Gezhou Dam on the Yangtze River, China (n=17) (this is a bottom-feeding species that migrates to the sea after hatching and does not return to rivers to breed until around 14 years old). Organs and eggs were harvested and samples were kept at −20 °C until analysis. Fish age was estimated by growth layers in the cleithrum.

Samples were spiked with $^{13}C_{12}$-PCB-180 prior to extraction, and analysis was by GC-MS with negative ionization. The recovery of the standard was 85±19 %, and the recoveries of dechlorane compounds ranged from 85 to 123 %. All equipment rinses were carried out with acetone and n-hexane to avoid sample contamination. A laboratory blank was incorporated in the analytical procedures for every batch of 12 samples. The LoD was 0.0002 µg/kg [2 ng/kg] ww for both isomers of Dechlorane Plus and DP-1Cl.

Both Dechlorane Plus isomers were found in all tissues, although comparisons between tissues is complicated by the varying sample numbers (egg, n=14; gonad, n=5; adipose, n=5; liver, n=7; heart, n=5; muscle, n=7; intestine, n=7; stomach, n=5; gill, n=6; pancreas, n=2; gallbladder, n=1; spleen, n=1; kidney, n=1), as well as the very low concentrations that were measured. Median (total) concentrations were 0.0063 µg/kg ww in eggs, 0.0056 µg/kg ww in gonads, 0.0094 µg/kg ww in adipose tissue, 0.0088 µg/kg ww in liver, 0.0135 µg/kg ww in heart, 0.0020 µg/kg ww in muscle, 0.0034 µg/kg ww in intestine, 0.0042 µg/kg ww in stomach, 0.0044 µg/kg ww in gill, 0.0136 µg/kg ww in gall bladder, 0.0048 µg/kg ww in spleen, 0.0078 µg/kg ww in kidney (only a minimum and maximum are given for each isomer in pancreas). The concentration in heart tissue was unexpectedly high even though the lipid content of the heart (4.4 %) was much lower than adipose tissue (66 %) and liver (13 %)[59]. Relatively high concentrations were also found in eggs compared to muscle.

The f_{anti} values were as follows (the paper appears to have a missing line for some tissues): eggs and heart, 0.58±0.03; stomach, 0.72±0.06; liver, 0.72±0.03; gonad, 0.68±0.03; intestine, 0.67±0.08; and egg 0.65±0.04. The f_{anti} values in eggs and heart were significantly lower than those in muscle and liver ($p < 0.001$).

Syn- and anti- isomers of DP-1Cl were detected in all tissues, with the highest concentrations in adipose (median concentrations were 2.7 ng/kg ww for syn-DP-1Cl and 6.6 ng/kg ww for anti-DP-1Cl), followed by heart (median concentrations were 2.5 ng/kg ww for syn-DP-1Cl and 2.5 ng/kg ww for anti-DP-1Cl). Anti-DP-1Cl had a different tissue distribution pattern to Dechlorane Plus, with levels in maternal tissues (including muscle, liver, intestine and stomach) statistically significantly lower than those of eggs.

The ratios of the concentration of substances in eggs to those in summed maternal tissues (used to assess maternal transfer efficiencies) were 3.7 and 5.2 for anti- and syn- isomers, respectively (and 5.2 and 2.6 for anti- and syn-DP-1Cl, respectively). Regression analysis was conducted to investigate age-related accumulation trends in eggs from individually aged fish. Statistically significant negative age-related accumulation trends were found for anti- and syn-DP-1Cl, while the relationship for the two Dechlorane Plus isomers was not statistically significant.

To evaluate the potential influence of maternal transfer on age-related decreasing concentration trends, the relative contributions of the substances in eggs to total body burden were calculated. Eggs contributed around 50% of the overall body burden for the syn- and anti-

[59] These are the values quoted in the text of the paper. Lipid contents reported in Table 1 of the paper appear to be in error, as the lipid content is given as 0.04 for adipose and 0.66 for liver. On that basis, no attempt has been made to estimate lipid weight concentrations.

isomers of Dechlorane Plus, approximately equivalent to muscle tissue (values read from a graph), reflecting not only their relatively high concentrations, but also their large size. Maternal transfer to eggs was considered to be a possible explanation for the apparent age-related decrease in some substance concentrations in female Chinese Sturgeon. It is unclear where in the life cycle the parent fish were exposed to Dechlorane Plus. They spend the first fourteen years of their lives at sea, before spending around a year in the Yangtze River and then returning to the sea for 3 to 5 years before spawning again.

Principle component analysis of concentrations of other substances in the same fish individuals showed that the dechlorane compounds (including Dechlorane Plus) separated from perfluorocarbons, organotins and hydroxylated PBDEs, which are thought to be protein-binding pollutants. This suggests that Dechlorane Plus is a lipid-binding compound. The paper does not provide lipid-normalized concentrations for Dechlorane Plus, and the no attempt has been made to estimate them due to potential reporting errors in the data table.

Finally, liver microsomes were isolated from cultured two-year-old Chinese Sturgeon with dithiothreitol (DTT) to preserve the catalytic activity of reductases. The protein concentration in the reaction vial was 2.4 mg/mL and the CYP1A1-catalyzed EROD activity was 4.6 pmol/mg/min. Treated microsomes were incubated in vitro at 37 °C for 24 hours with constant agitation, and incubations without chemicals and without microsomes were used as negative controls to assess background contaminants and the possibility of non-enzyme-mediated changes in chemical structure. The concentration of syn- and anti-Dechlorane Plus in the incubation mixture was 50 ng/mL [50 µg/L], which is significantly higher than the reported solubility in pure water. After incubation, the samples were extracted by n-hexane immediately for chemical analysis. No dechlorinated substances were detected indicating that the metabolism rates were very low in Chinese Sturgeon.

In summary, Dechlorane Plus can be found at low concentrations in a variety of tissues in an endangered species that spends large parts of its life at sea, including its eggs. The study found no evidence of dechlorination in liver microsomes *in vitro*. This study is not included in the registration dossier.

6. He *et al*. (2014) investigated bioaccumulation in fish from the Dongjiang River catchment in the Pearl River Delta in southern China. The lower river drainage contains numerous tributaries that run through Dongguan city, a major centre of electronic goods manufacture. The following samples were collected: surface sediment (top 5 cm layer, n=42) and a 76 cm deep sediment core (thought to represent about 15 years' deposition based on sedimentation rates) in July 2009, water (n=5) in May 2010, and three fish species (n=34) from different tributaries in September 2010 (Mud Carp (*Cirrhina molitorella*, n=9), Nile Tilapia (*Tilapia nilotica*, n=15), and Plecostomus (*Hypostomus plecostomus*, n=10)). Muscle tissue was excised in the laboratory. All the samples were stored at −20 °C prior to analysis. Following sample extraction, chemical analysis was performed by gas chromatography/electron capture negative ionization (ECNI) mass spectrometry.

   Dechlorane Plus was detected in both dissolved and particulate phases in the water samples at mean concentrations (total isomers) of 2.38 (range: 1.2 – 3.3) pg/L and 0.39 (range: 0.24 – 0.78) ng/L, respectively. The mean f_{anti} value in the particulate phase was 0.79 ± 0.01 (an f_{anti} value

was not estimated for the dissolved phase as the levels of the syn- isomer were very close to the detection limit). Both isomers were also detected in all surface sediment samples, at a mean concentration of 2.9 (range: 0.08 – 19.4) µg/kg dw. The f_{anti} value was 0.77±0.09, which is similar to the value in the technical product, suggesting that stereoselective degradation was limited. The mean concentration in the sediment core (split into 19 section) was 5.9 µg/kg dw, but Dechlorane Plus levels were significantly higher in the top layer of the sediment core (depth < 36 cm, 0.35 – 57.6 µg/kg dw) than in the lower section (0.02 – 0.72 µg/kg dw), indicating an increase in contamination in recent years. No clear trend in f_{anti} (increase or decrease along with depth) was found in the sediment core.

Dechlorane Plus was detected in 80% of the fish samples. The mean concentrations (total isomers) in the carp, tilapia and plecostomus were 15.8 (range: 'not detected' – 89.6) µg/kg lw, 20.6 (range: 'not detected' – 189.3) µg/kg lw and 15.9 (range: 'not detected' – 52.3) µg/kg lw, respectively. The mean f_{anti} values were 0.6 (range: 0.15 – 0.94), 0.59 (range: 0.19 – 0.93) and 0.71 (range: 0.54 – 0.88) for carp, tilapia and plecostomus, respectively. These were significantly lower than those in sediments and water (p < 0.05, t-test), suggesting enrichment of the syn- isomer in fish (implying stereospecific metabolism, uptake and/or elimination). The values for Plecostomus were higher than the other two species, and the paper notes that Plecostomus is thought to have a low capability for PBDE metabolism, which might possibly be relevant for other highly halogenated substances. The $\delta^{15}N$ of the Plecostomus (10 ± 3‰) was higher than those in the carp (6 ± 3‰) and tilapia (7.4 ± 0.9‰), so there was no clear trend in mean concentration with trophic position (this is not discussed in the paper).

Mean BSAFs (defined as the lipid normalized concentration in the fish divided by the organic carbon normalized sediment concentration) for total isomers were 0.047, 0.069 and 0.063 in the carp, tilapia and Plecostomus, respectively (N.B. the abstract quotes different BSAFs, i.e. 0.024, 0.037 and 0.10; the paper does not provide the organic carbon content of the sediment, so it is not possible to check which numbers are correct). The BSAFs for the syn- isomer were higher than those for the anti- isomer in all three species (with the exception of two samples). The BSAFs were similar to those for BDE-183 (mean of 0.049, 0.051 and 0.014 in the three fish species), lower than those of PBDEs with less than 7 bromine atoms) and higher than nona- and decaBDE congeners reported in another study by the same authors. This suggests that the bioaccumulation potential of Dechlorane Plus from sediments may be similar to that of BDE-183.

The study is considered to be reliable with restrictions for the purposes of this evaluation. The relationship between the sediment sampling sites and the home range of the fish is not stated, and there was a gap of 14 months between the collection of sediment and fish. In addition, it is unclear whether the lipid normalized muscle concentrations provide an appropriate representation of whole body concentrations in the fish. The reliability of the derived BSAFs is therefore uncertain.

This study is included in the registration dossier as supporting monitoring information and is considered fully reliable by the Registrant. Although the bioaccumulation aspects are not discussed in the CSR, the study summary does mention the low BSAFs and the apparent stereospecific metabolism of anti-DP and/or stereoselective uptake of syn-DP in fish.

7. Zhang *et al*. (2011b) investigated the influence of food source and trophic position on the levels of Dechlorane Plus in waterbirds (as part of a study of PBDE congener profiles). Specimens (n = 29) from five bird species found dead or dying from various causes (hunting, poisoning, distress, etc.) (Chinese Pond Heron *Ardeola bacchus* (n= 5); White-breasted Waterhen *Amaurornis phoenicurus* (n = 11); Slaty-breasted Rail *Gallirallus striatus* (n = 5); Ruddy-breasted Crake *Porzana fusca* (n = 5) and Common Snipe *Gallinago gallinago* (n = 3)) were collected between 2005 and 2007 from Qingyuan County, the second largest e-waste recycling region in the Pearl River Delta, south China. Pectoral muscle, liver and kidney were excised and all tissues were stored at -20 °C until analysis.

   Following sample extraction and clean-up, Dechlorane Plus was analysed using gas chromatography-mass spectrometry using negative chemical ionization (NCI) in the selective ion-monitoring (SIM) mode. The recoveries of 2,2,4,4,5-pentachlorodiphenyl ether (CDE-99) and ^{13}C-BDE-209, used as surrogate standards, were 87-113 % and 86-105 %, respectively. Five spiking blanks with Dechlorane Plus and five procedural blanks were performed. The average recoveries for anti- and syn-isomers were 109 and 102 %, respectively. The relative standard deviations of all targets were less than 5 %, and of duplicates (n = 3) were less than 15 %. The limit of quantification was 0.06 and 0.1 ng/g for the anti- and syn- isomer, respectively. Subsamples of pectoral muscle for nitrogen and carbon stable isotope analysis.

   Both Dechlorane Plus isomers were detectable in all of the species, except for one Chinese Pond Heron, with total concentrations ranging up to 610, 2 200 and 1 830 ng/g lw in muscle, kidney and liver, respectively. The highest levels were observed in Slaty-breasted Rail (muscle: 14 – 610; liver: 55 – 920; kidney: 21 – 830 ng/g lw). Generally, these concentrations were one to two orders of magnitude lower than the corresponding concentrations of total PBDEs, except for the Ruddy-breasted Crake, whose Dechlorane Plus concentrations were comparable to those of total PBDEs. No difference in the f_{anti} between tissues was found in a given species (by analysis of variance (ANOVA)) so an overall f_{anti} was calculated for each of the five species. The mean f_{anti} for the Chinese Pond Heron, White-breasted Waterhen, Common Snipe, Ruddy-breasted Crake and Slaty-breasted Rail were 0.34, 0.36, 0.43, 0.46 and 0.61, respectively. These values are all lower than the f_{anti} of the commercial substance (0.70), implying a preferential accumulation of the syn- isomer in biota.

   No significant difference (t test, $p > 0.05$) in the kidney and liver concentrations was detected in any species, regardless of whether the concentration was expressed on a wet or lipid weight basis (although the ratio varied greatly between less than 1 to larger than 1). Muscle concentrations were lower than liver concentrations for all bird species based on wet weight. The lipid contents in liver were higher than those in muscle, which may partially explain the high concentration in liver. When the concentrations were expressed on lipid weight, the ratios were still less than 1 for all bird species except the crake. This observation suggests that Dechlorane Plus accumulates to a lesser extent in bird muscle than liver, which might be important when considering studies that are based on pectoral muscle only.

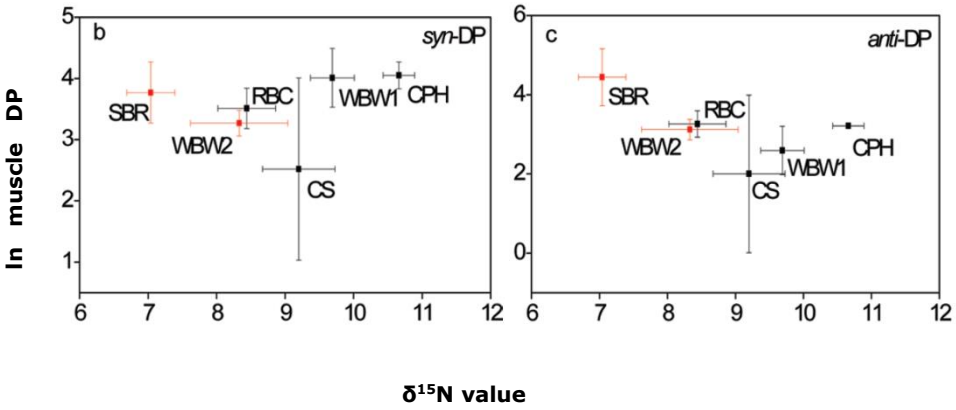
   The relative trophic status of the birds, defined by δ^{15}N, increased in the following order: Slaty-breasted Rail (7.0‰) < Ruddy-breasted Crake (8.4‰) < Common Snipe (9.2‰) and White-breasted Waterhen

(9.2‰) < Chinese Pond Heron (10.7‰). This was consistent with their different feeding habits. The heron feeds primarily on fish. The waterhen mainly eats seeds, insects and small fish, and often forages above ground in low bushes and small trees. The snipe feeds mainly on aquatic insects and invertebrates. The crake feeds mostly on plant material and some aquatic insects. The paper does not discuss the diet of the rail[60], but points out that unlike the other species, the rail samples had a relatively high $\delta^{13}C$ but low $\delta^{15}N$, suggesting a primary food source different from that of the other species. This appeared to be confirmed by differences in PBDE congener patterns, which also applied to a sub-sample of waterhens.

The relationship between trophic level and monitored Dechlorane Plus levels was examined by regressing the log-normalized concentration of the substance in muscle against $\delta^{15}N$ (excluding rail and the sub-sample of waterhens with an apparently different food source) (see Figure 3). Neither syn- nor anti-Dechlorane Plus correlated well with the $\delta^{15}N$ ($p > 0.05$), suggesting a lack of biomagnification.

This study is included in the registration dossier and is considered fully reliable by the Registrant. The sample size was relatively small, whole body concentrations (if available) could possibly lead to a different conclusion, and that there was no analysis of any food items of the sampled species. Conclusions about bioaccumulation potential are therefore difficult to draw, although it is notable that the substance was detected in all species, regardless of diet.

Figure 7: Relationship between $\delta^{15}N$ and Dechlorane Plus muscle concentrations in wild birds from South China



Note: Error bars represent ±1 standard error. CPH: Chinese Pond Heron; WBW1: White-breasted Waterhen (7 of 11); WBW2: White-breasted Waterhen (4 of 11); CS: Common Snipe; SBR: Slaty-breasted Rail; RBC: Ruddy-breasted Crake.

(reprinted with permission from Zhang *et al*. (2011b). Copyright 2011: American Chemical Society)

[60] http://www.hbw.com/species/slaty-breasted-rail-lewinia-striata indicates that the rail eats worms, molluscs, crustaceans, insects and their larvae, spiders, and the seeds and shoots of marsh plants.

8. Peng *et al*. (2014) investigated trophic magnification of Dechlorane Plus and DP-1Cl (both syn- and anti- isomers) (and other dechlorane substances) in a marine food web in Liaodong Bay in the northern region of the Bohai Sea, an enclosed inner sea in northern China (with an area of about 10 000 km^2 and a maximum depth of about 32 m).

   The sampling regime is reported in two separate papers (Zhang *et al*., 2010a; Zhang *et al*., 2012). Samples were collected in November 2006. Sediment samples (n=15) were taken from the subsurface zone (0−30 cm depth) in transects over a relatively wide area (the actual sample area is indicated on a map but no co-ordinates or distances are provided). In contrast the food web included five invertebrate and eight fish species collected by bottom trawl at a single location (40°42N; 121°46E), and two gull species (both adults and juveniles for one of them) trapped at a different location (40°52N 121°51E).

   With the exception of juvenile gulls, no information is provided in the paper or supporting information about the age of the sampled organisms. From the length and weight data, it appears that the individual invertebrate and fish species were broadly from the same age class, with the possible exception of Flathead fish and Black Spotfed Bass, for which the variation was much higher.

   All samples were stored at −20 °C prior to analysis. Whole soft tissues of invertebrates and muscles of fish and seabirds were used for the analysis. Following sample work-up, the concentrations of all substances in the extracts were determined relative to ^{13}C-labeled PCB180 by GC-MS (electron capture negative ionization). Recoveries of the target substances were 81−97 % for sediments, and 72−89 % for biota samples. Recoveries of ^{13}C-labeled PCB180 were 85 ± 19 % and 83 ± 24 % in sediment and biota samples, respectively. All equipment rinses were carried out with acetone and hexane to avoid sample contamination. A laboratory blank was incorporated in the analytical procedures for every batch of 12 samples. The method detection limits (MDLs) for Dechlorane Plus, which was detected in blank samples at "minor" concentrations, were set to be the three times of the mean concentration in the blank samples, and concentrations of the compounds were blank corrected. The MDLs for other compounds, which were not detected in blank samples, were set to the instrumental minimum detectable amounts with a signal-to-noise ratio of 3. The MDLs for the substances were 1.0 pg/g dw in sediments, and 0.08−2.0 pg/g ww for biota samples. Concentrations less than their respective MDLs were assigned a proxy value of half the MDL.

   Trophic levels were determined using stable nitrogen isotope ratios measured in invertebrate soft tissues (n=3 for each species) and muscle tissue for fish (n=3 for each species) and birds (n=16 for Black-tailed Gull, n=7 (*sic*) for Black-headed Gull). The stable nitrogen isotope ratios were 8.98 ± 0.44 to 12.5 ± 0.81 for invertebrates, 9.50 ± 1.0 to 14.1 ± 0.23 for fish, and 12.8 ± 1.4 to 14.2 ± 1.2 for gulls. The trophic enrichment factor was estimated to be 2.4−4.8‰ (average: 3.8‰) by examining isotopic differences between consumers and species that form their potential diets (such as Short-necked Clam and the crustacean *Upogebia major*). The study authors stated that the average enrichment factor corresponded well to that previously reported for other aquatic ecosystems.

   Short-necked Clams were used to estimate the δ^{15}N baseline and assumed to represent trophic level 2.0. The trophic levels calculated for

Small Yellow Croaker and Black Spot-fed Bass using an enrichment factor of 3.8‰[61] were 2.85 ± 0.13 and 3.36 ± 0.06, respectively, which were similar to those obtained from traditional stomach content analysis (2.99 and 3.37, respectively) in a previous study. Stable carbon isotope ratios ranged from −15.7 ± 0.09 to −17.9 ± 0.25 for invertebrates (except for *Mactra quadrangularis*: −12.6 ± 0.30), from −15.3 ± 1.1 to −17.2 ± 0.86 for fish (except for Goby: −12.7 ± 2.1), and from −18.6 ± 1.3 to −20.9 ± 2.7 for gulls.

All the substances were detected in sediments and biota (see

Table **7**). The mean sediment concentration was 6.7±16, 33±73 and 33±66 pg/g [ng/kg] dw for syn- and anti-Dechlorane Plus, and anti-[DP-1Cl], respectively.

The levels of the two Dechlorane Plus isomers were highly variable, but with higher average wet weight concentrations of the anti- isomer than the syn- isomer in many (though not all) species. Whilst this might possibly reflect species differences, it could also reflect the limited sampling and/or variability due to the low concentrations. Mean concentrations of the anti- isomer exceeded 100 pg/g ww only in the crustacean *Upogebia major*, China Anchovy and Black-headed Gull (in contrast, the highest mean syn- isomer concentration was 69 pg/g ww, in a fish species). The paper reports that the anti- isomer was the predominant one in sediment and invertebrates, with the contribution decreasing to 4.6−21 % in fish species at high trophic levels, and decreased to 2.8−4.4 % in seabirds. However, given the high variability and small sample numbers, this might be misleading.

According to the paper, the average concentrations of total Dechlorane Plus isomers were 817 (±1 030) pg/g lw in Black-tailed Gulls and 1 780 (±1 650) pg/g lw in Black-headed Gulls. An attempt has been made to replicate these calculations for the purposes of this assessment by dividing the reported mean wet weight concentrations by the mean percentage lipid content, but derived slightly different values (e.g. 1 875 pg/g lw for Black-headed Gull); the highest lipid weight concentration for both isomers combined was obtained for the Goby (10 900 pg/g lw).

The mean wet weight concentration of anti-[DP-1Cl] was lower than that of anti-Dechlorane Plus in all species (whereas the sediment concentration was similar), although it was nevertheless detected in 21 of the 23 gull samples. There was a significantly positive correlation between concentrations of anti-[DP-1Cl] and anti-Dechlorane Plus in gulls ($r^2 = 0.93$, $p < 0.001$). The study authors speculated that this may be due to natural accumulation of anti-[DP-1Cl] from sediments through the food web to gulls, or metabolism within gulls after accumulating Dechlorane Plus. However, an *in vitro* study failed to confirm the latter hypothesis (see Section 3.4.2.1). Significantly positive correlations for these two substances were also observed in sediments ($r^2 = 0.90$, $p < 0.001$) and China Anchovy ($r^2 = 0.80$, $p = 0.005$).

[61] $TL_{consumer} = 2 + (\delta^{15}N_{consumer} - \delta^{15}N_{\textit{Ruditapes philippinarum}})/3.8$, where $\delta^{15}N_{\textit{Ruditapes philippinarum}}$ was 8.98. For seabirds, the relationship was modified to $TL_{seabird} = 3 + (\delta^{15}N_{seabird} - \delta^{15}N_{\textit{Ruditapes philippinarum}} - 2.4)/3.8$.

Table 7: Concentrations of Dechlorane Plus in a Chinese marine food web (from Peng *et al*., 2014)

Species		Sample no.	Lipid content %	Trophic position	Concentration, pg/g ww		
					Dechlorane Plus		Anti-DP-1Cl
					Syn-	Anti-	
Invertebrates							
Short-necked Clam *Ruditapes philippinarum*		3	0.28± 0.08	2.00± 0.07	0.4±0.2	1.6±1.2	ND
[Bivalve mollusc] *Mactra veneriformis*		3	1.9± 0.09	2.05± 0.09	24±12	17±6.0	0.34± 0.18
Rock Shell *Rapana venosa*		3	0.30± 0.16	2.87± 0.09	0.4±0.2	1.8±1.2	ND
Chinese Mitten-handed Crab *Eriocheir sinensis*		3	0.64± 0.06	2.74± 0.14	0.4±0.2	2.6±1.8	ND
Mole Cricket (*sic*)[62] *Upogebia major*		4	1.50± 0.95	2.93± 0.12	2.8±2.1	112± 115	2.8±2.1
Fish							
Redeye Mullet *Liza hematocheila*		5	2.9±1.0	2.14± 0.15	3.7±2.3	7.6±6.3	0.21± 0.31
Goby *Synechogobius hasta*		5	0.78± 0.18	2.50± 0.14	51±28	34±41	5.7±6.0
Small Yellow Croaker *Pseudosciaena polyactis*		6	1.90± 0.38	2.85± 0.08	7.2±6.7	8.0± 10.0	0.45± 0.39
China Anchovy *Thrissa kammalensis*		10	9.80± 0.11	2.98± 0.06	21±11	145±91	21±15
Japanese Spanish Mackerel *Scomberomrus niphonius*		3	4.50± 0.27	3.01± 0.09	24±5.2	12±15	0.34± 0.18
Half-smooth Tongue-sole *Cynoglossus semilaevis*		6	1.40± 0.18	3.09± 0.04	69±96	60±79	1.7±3.2
Flathead fish *Platycephalus indicus*		3	1.23± 0.06	3.15± 0.04	ND	10±0.5	0.5±0.8
Black Spotfed Bass *Lateolabrax japonicas*		3	0.62± 0.06	3.36± 0.03	ND	7.8±3.0	0.4±0.8
Birds							
Black-tailed Gull *Larus crassirostris*	Adult	12	6.4±0.1	3.62± 0.22	10±10	58±60	29±32
	Juvenile	5	6.7±0.2	3.37± 0.38	1.8±1.1	10±14	3.9±4.2
Black-headed Gull *Larus ridibundus*		6	7.20± 0.53	3.78± 0.10	17±13	118± 128	56±54

Note: ND – not detected in any sample. All values were indicated by mean ± SD.

The concentration of syn-DP-1Cl was below the MDL in more than 30% of samples (data are not reported in any detail the paper); the highest concentration of syn-DP-1Cl was highest in seabirds, at 0.8 ± 2.2 pg/g ww, although it was only found in three Black-tailed Gull samples).

After adjusting for total organic carbon (TOC)[63] and lipid content, the biota-sediment accumulation factors (BSAFs) for birds were in the range 0.05 – 0.15 for syn-Dechlorane Plus, 0.08 – 0.25 for the anti- isomer and 0.05 – 0.12 for anti-[DP-1Cl]. These were much lower than those of

[62] This is the English name provided in the paper. From an Internet search, the Latin name indicates a marine crustacean, sometimes called Japanese Mud Shrimp.

[63] Sediment TOC was 0.48 ± 0.04%.

PBDEs in the same gull samples (BSAF >25 for total PBDEs). Although not considered in the paper, the BSAF for one species (*Upogebia major*) was above 1.

Both gull species are resident in Liaodong Bay, and feed mostly on insects, crustaceans and fish. The China Anchovy was assumed to be the major food item for the birds (from the fish species sampled) based on previous studies and also similar stable carbon isotope ratios. Trophic level-adjusted biomagnification factors (BMF_{TL})[64] were estimated for the gull–China Anchovy feeding relationship, as 0.14 – 0.34 for syn-Dechlorane Plus, 0.11 – 0.33 for the anti- isomer and 0.36 – 0.70 for anti-[DP-1Cl]. However, there is no information about the proportion of the diet that this fish species comprises for the gulls, and the amount of accumulation in the birds would also reflect sources in other areas during their lifespan.

Fourteen species were included in the TMF calculation[65] (excluding *Mactra quadrangularis*, Goby and juvenile gulls on the basis of different carbon isotope ratios; it is not clear why juvenile gulls would be feeding on a different carbon source to the adult birds). Although TMFs above 1 were estimated for both Dechlorane Plus isomers, these were not statistically significant: TMF values were 2.2 for the syn- isomer (r^2 = 0.05, p = 0.43) and 1.6 for the anti- isomer (r^2 = 0.03, p = 0.54). The data for the anti-isomer are presented in Figure 4 as an example.

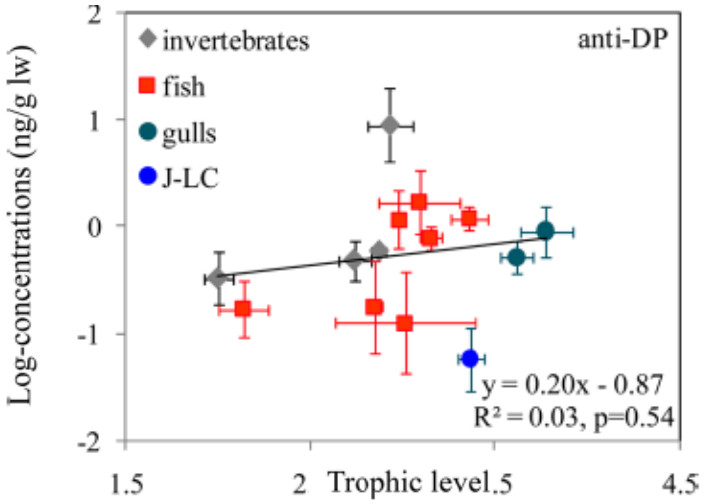
The TMF of anti-[DP-1Cl] was statistically significant, and estimated to be 5.6 (r^2 = 0.52, p = 0.004) when birds were included, or 4.1 (p = 0.05) when birds were excluded. A TMF was not calculated for syn-DP-1Cl due to the high number of non-detects.

The study authors concluded that Dechlorane Plus did not biomagnify in this marine food web (believed to be due to low bioavailability), whereas biomagnification was observed for anti-[DP-1Cl]. However, sample numbers were very small, particularly for invertebrates and fish, and the measured concentrations were also relatively low in this marine environment (e.g. compared to studies from the Great Lakes region of North America, gull concentrations were at least an order of magnitude lower). Whole body concentrations were not reported (muscle only for fish and birds). Measured concentrations were also highly variable in many species and non-detects were assumed to be half the detection limit value, which could bias the mean concentrations in some cases. In addition, the relationship between the sampled species and sediment is uncertain (e.g. due to small and larger scale migratory movements).

[64] Using the equation $BMF_{TL} = 10^{[\log10(([gull]/[prey])/(TLgull\ TLprey))]}$ where [gull] and [prey] are the lipid corrected concentrations of the substance in gulls and China Anchovy, respectively, and $TL_{gull/prey}$ is the trophic level for each.

[65] TMF was estimated by plotting the logarithm of the substance concentrations on a lipid basis against trophic level. The TMF was 10 raised by the slope of this plot.

Figure 8: Regressions between trophic level and concentrations of anti-Dechlorane Plus in aquatic species in a Chinese marine food web (error bars represent ± 1 standard error)



Note: J-LC = juvenile Black-tailed Gull

(reprinted with permission from Peng *et al*. (2014). Copyright 2014: American Chemical Society)

Overall, the study is of limited usefulness as the representivity of the data is not known, and the reliability of conclusions based on mean concentrations is highly uncertain. Instead it should be noted that:

a) Dechlorane Plus was found in all species in this marine food web, despite the presumably high dilution available in the water column.

b) The highest lipid weight concentration of both isomers combined was found in a fish species (Goby) that did not appear to be feeding on the same carbon sources as the other species in the food web.

c) There appears to be a trend of increasing concentrations of the anti-isomer with trophic level in fish species (see Figure 4).

d) Juvenile gulls contained lower amounts than adults, suggesting that longer-term exposure can lead to higher body burdens. The gulls contained average concentrations of total Dechlorane Plus isomers of around 1 - 2 µg/kg on a lipid weight basis.

This study is not included in the registration dossier.

9. Wang *et al*. (2015) investigated trophic magnification of Dechlorane Plus and three potential degradation products (anti-DP-1Cl, anti-DP-2Cl and DPMA) in an aquatic food chain that receives discharges from the Dechlorane Plus production facility in Huai'an, Jiangsu province, China. Samples of seven aquatic species (two invertebrates and five fish) were collected concurrently from the Beijing-Hangzhou Grand Canal in May 2010[66]. No information is provided in the paper or supporting information

[66] Wang *et al*. (2010) reported a Dechlorane Plus concentration of 8.0 µg/kg dw in a surface sediment sample collected from this canal within 0.5 km of the Chinese manufacturing site (although this appears

about the age of the sampled organisms. Composite samples were used, comprising five individuals of each species (two for shrimp).

All samples were stored at −20 °C prior to analysis. Whole soft tissues of invertebrates and fish muscle were used for the analysis. Following sample work-up, the concentrations of all substances in the extracts were determined by GC-HRMS analysis operated in electron ionization (EI) mode, with all compounds identified with retention times ±0.10 min of the calibration standard, and verified using the two most abundant ions of the fragment cluster, corresponding mass ions ratios, and signal–noise ratio (>3). The surrogate and internal standards used for all compounds were 2,20,4,40,6,60-hexachlorobiphenyl (CB-155) and octachloronaphthalene. Calibration standards were prepared in the range of 1–50 ng/mL except for Dechlorane Plus isomers (1–50 000 ng/mL). CB-155 surrogate standard recoveries ranged from 52 % to 85 % and the final concentrations were corrected using the surrogate standard recoveries in all samples. Laboratory procedural blanks were used to monitor background contamination from sample handing and processing. Procedural blanks were extracted and analyzed identically to the samples. A procedural blank was run for every 12 samples. Only trace levels of Dechlorane Plus isomers were detected in the procedural blanks. The limits of quantification (LOQ) were calculated as 10 times signal-to-noise ratios (and were below 1 ng/g [µg/kg] lipid weight (lw) for all test compounds).

Stable nitrogen isotope ratios were measured to determine trophic positions. The trophic level of each sample was calculated from $\delta^{15}N$ by using an enrichment factor of 3.4‰ relative to the primary consumer (snail), which was assigned a trophic position of 2.

All the substances were detected in sediments and biota (see Table 8).

Although not stated in the paper, total Dechlorane Plus wet weight concentrations were in the range 0.8 – 1.1 mg/kg for four of the five fish species (i.e. all except Loach). The paper indicated that these levels reflected the point source contamination at this site (unlike the majority of other studies where exposure is more diffuse).

All TMFs ranged between 1.0 and 3.1, although the relationship was only statistically significant for anti-Dechlorane Plus (TMF = 1.9 (95 % CI: 1.1–3.4), $r^2 = 0.11$, $p = 0.04$) and total Dechlorane Plus isomers (TMF = 2.2 (95 % CI: 1.2–3.9), $r^2 = 0.16$, $p = 0.01$), the latter driven by the data for the anti- isomer. This suggests that the substance can biomagnify up the food chain (no correlation was found between trophic level and lipid content, suggesting that biomagnification was not attributable to lipid content effects; the paper does not provide the supporting calculations). The plot of concentration against trophic level is given in Figure 5.

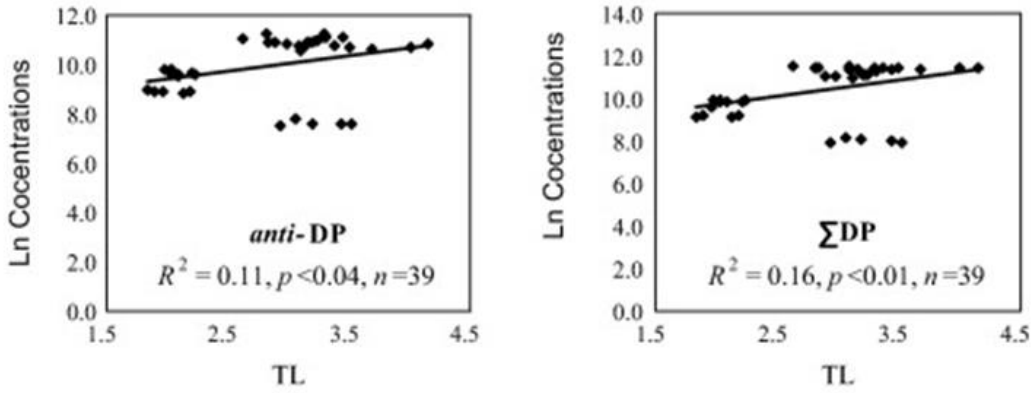
to have been upstream of the plant's wastewater outfall). It is not known how this site relates to the sampling location for the fish.

Table 8: Concentrations of Dechlorane Plus and potential degradants in a Chinese freshwater food web (from Wang *et al*., 2015)

Species	Sample	Lipid content, %	Trophic level	Concentration, ng/g lw					
				Dechlorane Plus			Anti-DP-1Cl	Anti-DP-2Cl	DPMA
				Syn-	Anti-	Total			
Invertebrates									
River snail *Viviparus sp.*	25	0.6	2.0± 0.2	3200± 2700	7300± 200	10500± 2600	126± 12	12.7 ±1.9	31.4 ±7.8
Freshwater shrimp *Macrobrachium nipponense*	14	1.03	2.1± 0.1	3700± 160	15500 ± 1900	19300± 500	238± 33	100± 13	17.3 ±5.8
Fish									
Crucian Carp *Carassius auratus*	6	1.26	3.1± 0.1	12000 ±2800	52400 ± 5900	64400± 3700	860± 90	165 ± 15	29.2 ± 4.5
Common Carp *Cyprinus carpio*	7	1.32	3.2± 0.1	20400 ±6600	66700 ± 10700	85700± 300	2340 ±410	6.03 ± 0.96	14.2 ± 3.1
Bleeker *Pseudolaubuca sinensis*	7	1.03	3.2± 0.4	45500 ±4500	47500 ± 7800	93000± 5600	2470 ±430	25.6 ±8.0	43.6 ± 9.7
Loach *Misgurnus anguillicaudatus*	5	2.06	3.3± 0.2	968± 244	2040± 190	3010 ± 330	144± 31	49.1 ±14.5	13.3 ± 4.9
Northern Snakehead *Channa argus*	2	0.95	4.1± 0.1	45400 ±4500	47200 ± 6080	92600± 1600	1010 ±50	232 ± 15	65.5 ± 14.8

Note: All values were indicated by mean ± SD.

Figure 9: Regressions between trophic level and concentrations of Dechlorane Plus (anti- and total isomers) in aquatic species



(reprinted with permission from Wang *et al*. (2015). Copyright 2015: Elsevier Ltd.)

A distinct difference between levels in invertebrates and the majority of the fish is apparent. The paper does not discuss whether the sampled species are part of the same food web, and the separation of the five lowest data points for fish (presumably for Loach; individual data are not provided) is striking (as is the range of trophic levels apparently covered

by this species). Loach live in sediments and leaf litter and eats worms, small crustaceans, insects/insect larvae, and other small aquatic organisms[67]. Shrimp at least would seem to be form part of the diet of this species.

The number of data points (n=39) indicates that the analysis was performed using individual fish measurements, along with five pooled groups of snail and seven pooled groups of shrimp, rather than species means. When species means are used, a TMF of 2.5 is calculated for the anti- isomer when the data for Loach are excluded.

In addition, the log-normalized concentration ratios of anti-DP-1Cl to anti-DP showed a significant relationship with trophic level ($p < 0.001$), implying that anti-DP-1Cl may have a higher trophic magnification potential than anti-DP.

The fish species occupying the highest trophic position had the lowest f_{anti} value (0.51), whereas the shrimp occupying the lowest trophic level had the highest f_{anti} value (0.81). A significant negative correlation was found between f_{anti} and trophic level for the sampled species ($p < 0.01$), suggesting that the organisms have lower uptake efficiencies and higher depuration rates for the anti- isomer compared to the syn-isomer.

Using sediment concentrations from an earlier study (Wang *et al*. (2010) – see Appendix 1) and an organic carbon content of about 2 %, BSAFs for anti-DP were estimated as 0.6 for Loach, 1.6 for Crucian Carp and 3.2 for Northern Snakehead, indicating an enrichment from food intake for the latter species. The BSAFs for syn-DP were lower than 1.7 for Common Carp, River snail, shrimp and Loach, indicating these species can accumulate syn-DP through exposure to sediment and water. However, the BSAFs for Northern Snakehead (5.3), Crucian Carp (9.0) and Bleeker (5.1) suggesting a role for dietary intake.

The study is limited by the small sample numbers involved. However, it indicates that point source emissions can lead to relatively high concentrations of Dechlorane Plus in fish, and that at least the anti-isomer can biomagnify.

This study is not included in the registration dossier.

10. Barón *et al*. (2013) investigated the concentrations of Dechlorane Plus in an aquatic food web from an industrial area of the south central Chilean coast. Samples were collected during July and September 2010 from the Lenga estuary (based on a previous sediment survey in February 2010 to identify an area with a high contamination level). It was not possible to collect all the biota samples in the same location, so samples were collected from a nearby area called Chome and Perone. This area receives industrial discharges from a large petrochemical complex.

    Fifty-five biota samples were collected (divided into three trophic groups based on diet), freeze dried and stored in sealed containers at −20 ºC until analysis. Lyophilized and homogenized samples were spiked with the surrogate standard ^{13}C-syn-DP before pressurized liquid extraction. Analysis was via GC/MS triple quadrupole, working in negative chemical ionization mode. Recoveries were in the range 82 – 97 %. Identification and confirmation was based on simultaneous responses to the two selected ions or transitions, a signal area at least three times higher than

[67] Details from http://www.fishbase.org/summary/Misgurnus-anguillicaudatus.html, accessed June 2015.

the signal noise, and the difference in the relative intensity of a peak with respect to theoretical values obtained with standard solutions not exceeding ± 15 %. Five procedural blanks were made to prevent interferences and contamination. The detection limit was 5.5–21 pg/g [ng/kg] lw and the limit of quantification was 7.7–70 pg/g lw. Results are summarised in Table 9.

Table 9: Concentrations of Dechlorane Plus in a Chilean marine food web (from Barón *et al*., 2013)

Species	No. of sample pools analysed (sample nos./pool)	Dechlorane Plus concentration, ng/g [µg/kg] lw			Detection frequency, %
		Syn-	Anti-	Total	
Primary consumer					
Giant Barnacle *Austromegabalanus pstittacus*	7 (3-5)	ND–0.3	ND–0.3	ND–0.6	71
Keyhole Limpet *Fisurella sp.*	3 (1)	0.8–2.9	1.5–7.0	2.3–9.8	100
Sea Squirt *Pyura chilensis*	6 (15-20)	ND–0.1	ND–0.2	ND–0.3	83
Clam *Venus antiqua*	6 (3)	ND–0.2	ND–0.3	ND–0.4	75
Razor Shell Clam *Tagelus dombeii*	7 (5)	ND–0.5	ND–5.4	ND–5.8	85
Secondary consumer					
Crab *Homalaspis plana*	1 (1)	ND	ND	ND	0
Crab *Taliepus dentatus*	4 (3-4)	0.1–0.4	0.1–0.8	0.3–1.1	100
Peruvian Morwong *Cheilodactylus variegatus*	2 (1)	0.04	0.04–0.1	0.08–0.1	100
Damselfish *Chromis crusma*	4 (1)	0.01–0.1	0.03–0.2	0.04–0.3	100
Tertiary consumer					
Sandperch *Pinguipes chilensis*	13 (1)	ND–0.9	ND–1.2	ND–2.0	69
Chilean Abalone *Concholepas*	4 (1)	ND	ND	ND	0

Note: ND – not detected in any sample.

Dechlorane Plus was detected in all of the primary consumer species (though not every sample), at a maximum concentration of 9.8 µg/kg lw (total isomers). It was also detected in all of the samples of secondary consumers, with the exception of one crab sample, with a maximum concentration of 1.1 µg/kg lw (total isomers). It was not detected in any sample of one of the tertiary consumer species (Chilean Abalone) but occurred at a maximum concentration of 2.0 µg/kg lw (total isomers) in the other species (Sandperch). Although the maximum concentration in tertiary consumers was higher than secondary consumers, it was lower than that in primary consumers, suggesting overall biodilution across the whole food web. Chilean Abalone is known to prey on Giant Barnacle and Sandperch, and Peruvian Morwong can feed on Keyhole Limpet; BMFs were below 1 for all three predator-prey relationships.

Sample numbers were low, and the organisms were not all sampled from the same locality or necessarily the same food chains, so the representivity of the data is unknown. F_{anti} values were similar for primary and secondary consumers (0.60 and 0.61 respectively), but lower for the tertiary consumers (0.47), which was significantly different (t-test, $p < 0.01$). This may indicate that syn-DP is more bioaccumulative than

the anti- isomer, or that tertiary consumers can eliminate the anti-isomer more efficiently.

This study is not included in the registration dossier.

11. She *et al*. (2013) evaluated the bioaccumulation of Dechlorane Plus in a short herbivorous food chain (paddy soil – rice plant *Oryza sativa* - apple snail *Pomacea canaliculata*) from an electronic waste recycling site in South China. Soil (n = 12; 6 pooled samples), tender rice plant leaves (n = 30; 10 pooled samples), and snails (n = 98; 12 pooled samples) were collected concurrently in 2010 from a paddy field. The snails were picked by hand, and the plants and soil were sampled from around the same location using stainless scissors and stainless steel shovels, respectively. All the samples were wrapped with aluminum foil, put in polythene zip-bags, and transported to the laboratory within 12 hours. At the laboratory, plant leaves were rinsed three times with distilled water and blotted with organic chemical free tissue paper. Soft tissue was dissected from the snails. All the samples were freeze-dried, ground with a mortar, and stored below 0 ºC until analysis.

    Samples were spiked with surrogate standards (BDE-77 and -181) and extracted, and the extracts spiked with known amounts of internal standards (BDE-118 and -128) prior to instrumental analysis via GC-MS using ECNI in SIM mode. Ions m/z 653.8 and 655.8 were monitored for Dechlorane Plus. Procedural blanks contained traces of the target chemicals, but the levels were less than 1 % of the analyzed concentration in the samples. The mean recoveries in spiking blanks were in the range 98 – 102 %. The recoveries of surrogate standards were 93 ± 2 % (mean ± standard error) for BDE-77 and 95 ± 2 % for BDE-181. No surrogate or blank corrections were made to the final concentrations. Target chemicals detected in the triplicate samples were consistent. Instrumental quality control was performed by regular injection of solvent blanks and standard solutions. The limits of quantification (LoQ) were in the range 0.01 – 0.22, 0.01 – 0.06, and 0.02 – 0.35 µg/kg dw for soils, rice plant, and apple snails, respectively.

    Dechlorane Plus was detected in all matrices, with an overall detection frequency of 96 % (although the ranges quoted in the paper imply that no measurement was below the LoQ). The mean concentrations for the syn- isomer were 3.66 (range: 2.89 – 4.52) µg/kg dw in soil, 0.23 (range: 0.16 – 0.39) µg/kg dw in rice plant leaves and 1.12 (range: 0.24 – 3.19) µg/kg dw in snails. The mean concentrations for the anti- isomer were 10.8 (range: 9.20 – 12.9) µg/kg dw in soil, 0.89 (range: 0.55 – 1.60) µg/kg dw in rice plant leaves and 3.06 (range: 0.99 – 6.35) µg/kg dw in snails. The mean total Dechlorane Plus concentration in the paddy soils was 14.5 µg/kg dw. The lipid content of the plant leaves and snails was 0.74 (range: 0.61 – 0.92) and 0.71 (range: 0.54 – 0.94) per cent dry weight, respectively.

    The mean plant-to-soil concentration ratio (defined as the ratio of the lipid-normalized concentration in the rice plant leaves to the organic carbon-normalized concentration in the paddy soil) was 0.05 (range: 0.03 – 0.11) and 0.07 (range: 0.04 – 0.15) for the syn- and anti- isomer, respectively. It should be noted that these ratios may not accurately represent the bioavailability of the substance, because measurements in other plant tissues (e.g. root) were not performed. In addition, as the plants were growing, it is unlikely that equilibrium had been achieved.

Mean biomagnification factors (defined as the ratio of the lipid-normalized concentrations in snails and in rice plants) were 3.1 (range: 0.63 – 7.9) and 2.3 (range: 0.59 – 4.7) for syn- and anti-Dechlorane Plus, respectively. Although rice plants are likely to be a major part of the diet of the snails in this study, *P. canaliculata* is a freshwater mollusc with gills, and so exposure via the paddy water is also a possibility (and this was not sampled). Nevertheless, this study implies that biomagnification can occur from rice plants to snail.

This study is not included in the registration dossier.

12. Jia *et al*. (2011) investigated concentrations of Dechlorane Plus in a coastal environment around Dalian, north-east China. The study involved sampling at 15 sites, representing one industrial, two urban and twelve rural locations (eight from the relatively enclosed Bohai Sea and seven from the more open Huanghai Sea) between October and December 2008. At least 15 individual oysters (species not stated) (n = 45[68]), and one water and one sediment sample were collected per site (n = 15 for both matrices) (the abiotic samples comprised five well-mixed subsamples collected from different locations at the site). It is not stated whether the oysters were allowed to depurate prior to analysis (i.e. it might be possible that some of the oysters contained the substance bound to particulates).

    At least one isomer of the substance was detected in most (≥78 %) samples (the limit of detection was 0.01 ng/g ww for each isomer in oysters). The mean total isomer concentration was 1.8 ng/L, 2.9 ng/g dw and 4.1 ng/g ww in water, surficial sediment (top 0-5 cm) and oyster tissue, respectively. The maximum concentration in oyster tissue was 8.1 ng/g ww for the syn- isomer and 11 ng/g ww for the anti-isomer (17 ng/g ww for total isomers). Isomer ratios suggested an enrichment of the syn- isomer in oyster.

    A statistically significant correlation (r^2 = 0.64, p < 0.001) was observed between total Dechlorane Plus concentrations in oyster and oyster sample lipid content. BSAFs (based on organic matter- and lipid-normalized concentrations) were calculated for paired sediment and oyster samples for which concentrations were above the detection limit. The mean BSAF for total isomers was 4.6 (range: 1.0 – 7.9). This value cannot be directly compared to BSAFs reported in other studies that use the sediment concentration normalized to organic *carbon* content. Organic *matter* concentrations are typically higher than the organic carbon content by a factor of around 2 (depending on matrix). The average BSAF based on organic carbon content would therefore be approximately 2.3.

    Although not presented in the paper, a BAF of 2 300 L/kg can be calculated from the average concentration in water and oysters.

    This study is not included in the registration dossier.

13. Shen *et al*. (2011a) investigated levels of Dechlorane Plus in suspended matter, sediment and Lake Trout (Latin name not provided, but presumably *Salvelinus namaycush*) in Lake Ontario, Canada. Niagara River suspended sediments were collected near the river mouth at

[68] Although not stated in the paper, presumably three pools of around five oysters were analysed per sampling site.

Niagara-on-the-Lake, Ontario using a large volume 24-h time-integrated dissolved/particulate phase sampler on a weekly/biweekly basis. Lake trout were collected every four to six years (1979, 1983, 1988, 1993, 1998 and 2004, with four or five individuals per time point) from Lake Ontario north of Main Duck Island as part of a long-term monitoring project. All samples were archived, and the temporal study was conducted on spring composites of these prepared from April and/or May samples from 1980 to 2002. Samples collected over the period April 2006 to March 2007 were combined into monthly composites to examine seasonal variation. Homogenates of individual whole fish were used. A sediment core was also collected from Lake Ontario using a mini-box core sampler followed by subsampling using push cores and extrusion and sectioning into 1 cm (for first 15 cm) and 2 cm intervals. A replicate core from the same box was dated by determining the ^{210}Pb activity as a function of the chronological age of the sediments.

Method detection limits were 0.5 - 1 pg/g dw in sediments and 5 – 15 pg/g lw for fish. Only low levels of the two Dechlorane Plus isomers were detected in procedural blanks. Mass-labelled surrogates for Dechlorane Plus were not added as the limited archived samples were originally analyzed for PCBs and dioxin/furans prior to study conception. Instead, spike tests confirmed that the substance was extracted, cleaned and fractionated quantitatively in a similar manner to the PCBs in sediments and fish tissue (e.g. recoveries in sediment samples were 102±4 % and 82±5 % for the syn- and anti- isomers, respectively). Reported concentrations were therefore not corrected for recoveries.

Total Dechlorane Plus concentrations (5.4±9.5 ng/g dw) were similar to those of BDE-209 in suspended river sediments collected since 2000, whilst the average concentration in the top 3 cm of the Lake Ontario sediment core (110 ng/g [µg/kg] dw) was greater. Both Dechlorane Plus isomers were also detected in all fish samples between 1979 and 2004, with an average concentration of 180±1 900 pg/g [ng/kg] lw.

The paper reports BSAFs calculated as the ratio of the average contaminant concentration in biota (wet weight) (for 1998 and 2004) and the corresponding average concentration in surface sediment (dry weight) (top 3 cm) from three sites in Lake Ontario using data from this study and Shen *et al*. (2010). The values were 0.8 and 0.3 for syn- and anti- isomers, respectively. For comparison, the corresponding BSAF for BDE-183 using this method was also 0.3. As for the study of Jia *et al*. (2011), these values cannot be directly compared to BSAFs reported in other studies that use organic carbon content-/lipid-normalized concentrations. The sample numbers were low, and the relationship between the sampled fish and sediment sites is unclear, so the reliability of the BSAF values is unknown.

The paper also notes that the fraction of the syn- isomer in the fish samples was higher than that reported in technical products, suggesting enrichment.

This study is included in the registration dossier and is considered fully reliable by the Registrant. The same information is presented in Shen *et al*. (2011b).

14. Wang *et al.* (2012) investigated the accumulation of Dechlorane Plus in a riverine environment in China. Samples of water (n=16), sediment (n=16), reed (*Phragmites australis*) (n=5, five sub-samples per site)

and Eelpout (*Enchelyopus elongatus*) (n=15) were collected from sampling sites along the Daling River in August 2010. The fish samples were collected from a single site in the river estuary. Analysis was by GC-MS, and strict quality assurance and quality control measures were taken to monitor the analytical process.

Dechlorane Plus was detected in all biota samples. The mean concentration (total isomers) was 0.63 (range: 0.53–0.88) µg/kg ww in reed and 29 (range: 8.7–93) µg/kg lw in fish. Although both isomers were measured in water and fish at the estuary site, dissolved concentrations were not measured, so a BAF was not estimated in the paper (total water concentrations are not reported for individual sites either).

The mean BSAF based on fish wet weight and sediment dry weight was 0.88 (range: 0.33–2.8) for the syn- isomer and 0.33 (range: 0.086–1.0) for the anti- isomer. The mean BSAF based on lipid and organic carbon normalised concentrations was 0.75 (range: 0.27–2.5) and 0.28 (range: 0.070–0.89) for the syn- and anti- isomers, respectively. The representivity of the sampling regime for the sediment at this site is unknown (it appears to have involved single grab samples and the estimated BSAF may have also considered sediment from areas other than those where the biota were sampled).

The mean f_{anti} in water, sediment, reed and fish was 0.72, 0.75, 0.73 and 0.53, respectively. The value for fish was significantly different from the other media ($p < 0.01$), suggesting enrichment of the syn- isomer.

This study is included in the registration dossier and is considered fully reliable by the Registrant.

15. Chen *et al*. (2012b) investigated levels of a variety of flame retardants in eggs from four gull species breeding across Canada. Eggs from fifteen Herring Gull (*Larus argentatus*) colonies in the Great Lakes region were collected from late April to early May 2008 (10-13 eggs per site, subsequently pooled). Individual eggs (n=10) from different nests of Glaucous-winged (*L. glaucescens*) (two sites), California (*L. californicus*) (one site), Ring-billed (*L. delawarensis*) (one site) or Herring Gulls were also collected from early May to early July 2008 from additional colonies across the country from the Pacific to the Atlantic.

Egg homogenate was ground with diatomaceous earth, spiked with internal standards (BDE-156 and ^{13}C-BDE-209), and then extracted with 50:50 dichloromethane:hexane using an accelerated solvent extraction system. The extract was subjected to gel-permeation chromatography, followed by clean-up on a silica gel cartridge. Analysis was by GC-MS in electron capture negative ionization (ECNI) mode using selected ion monitoring. Method blanks were processed to monitor interferences and contamination. A Standard Reference Material (SRM) was analyzed with each batch of samples (PBDE concentrations were within 8 ± 4 % of previous results). Replicate analyses (n=4) were made on standard additions to the SRM to evaluate the analytical precision and accuracy for Dechlorane Plus. Recovery efficiencies were comparable between non-PBDE substances and the internal standard BDE-156 (95 ± 13 %). Reported analyte concentrations were therefore corrected based on the recovery of BDE-156. Within individual colonies, none of analytes had wet weight-based concentrations significantly correlated with lipid content in individual egg homogenates ($p > 0.05$). The LoQ and LoD were

0.12/0.03 and 0.03/0.01 for the syn- and anti- isomers, respectively. For measurements below the LoQ, a regression probability plotting method was applied to assign values for statistical analysis.

Dechlorane Plus (total isomers) was frequently detected in eggs of all four species, with a maximum concentration of 5.5 µg/kg ww (for comparison, the maximum concentration of hexabromocyclododecane detected was 12 µg/kg ww). Only egg homogenate from one relatively pristine site (for Glaucous-winged Gull) did not contain the substance above the LoQ.

The gull colonies were located in a variety of freshwater and marine ecosystems. However, gulls tend to be migratory, feed opportunistically on a variety of aquatic and terrestrial food types, and birds from the same colony may consume different foods. This was reflected in large variations in egg stable isotope ratio values within colonies: egg $\delta^{15}N$ and $\delta^{13}C$ values were in the range 8.88 to 16.42‰ and -24.39 to -17.03‰, respectively.

A significant negative correlation between the concentration of Dechlorane Plus (total isomers) and $\delta^{15}N$ in Gull Island and Sable Island (Atlantic Ocean) gull eggs suggests that a greater reliance on a marine diet was related to lower Dechlorane Plus exposure. $\delta^{15}N$ values were not significantly correlated with total Dechlorane Plus levels at any other colony. No significant correlations of $\delta^{15}N$ to total PBDE concentration were observed at any colony.

Inter-colony comparisons of trophic position based on raw $\delta^{15}N$ data are inappropriate since the baseline $\delta^{15}N$ values for each colony are unknown. Each colony was therefore grouped into one of three ecosystem types depending on location (marine, Great Lakes and 'other freshwater'). There were also significant differences between colonies of Herring Gull and the other gull species in terms of total Dechlorane Plus concentrations, presumably reflecting differences in diet, migratory habits, trophic status and potentially bioavailability and elimination capacity. For example, Herring Gull and Ring-billed Gull eggs from Ile Deslauriers differed significantly in $\delta^{15}N$ and $\delta^{13}C$ values. To avoid complications due to inter-species differences in exposure pathways, subsequent analysis was based on the Herring Gull data. Statistically significant differences among ecosystems were found for total Dechlorane Plus levels ($p < 0.01$) in Herring Gull eggs, with marine colonies having significantly lower Dechlorane Plus concentrations than the freshwater ecosystems (the levels were not significantly different between the two freshwater ecosystems). PBDE patterns in eggs from different colonies indicated that exposure varied greatly, with freshwater colonies subject to elevated exposure to more highly brominated PBDEs. A non-significant positive correlation was observed between egg total Dechlorane Plus level and human population density ($r = 0.38$, $p < 0.079$), whereas no correlation was observed for hexabromocyclododecane.

The results of this study indicate that gulls can clearly accumulate the substance, but do not provide any evidence of trophic magnification.

This study is not included in the registration dossier.

16. Muñoz-Arnanz *et al*. (2012) collected eggs of Yellow-legged Gull (*Larus michahellis*) and Audouin's Gull (*Larus audouinii*) (n=19 per species)

from a breeding colony at the Chafarinas Islands in the south-western Mediterranean Sea during the 2007 breeding season.

The analytical method used was GC-MS-ECNI and the limit of quantification (LoQ) of the analytical method was 0.004 µg/kg for syn-DP, 0.009 µg/kg for anti-DP and ~0.003 µg/kg for the dechlorinated products anti-[DP-1Cl] and anti-[DP-2Cl]. DPMA was also included in the study but the recovery of this substance was very low (<10%) and so the results for DPMA are not considered further. Both stereoisomers were found above the LoQ in all the samples. Low levels were also found in most procedural blank samples and so the concentrations reported were blank-corrected.

Yellow-legged Gull eggs showed the highest average total Dechlorane Plus concentration (0.209 µg/kg ww, or 2.7 µg/kg lw), with an overall range of 0.0395 – 0.433 µg/kg ww (or 0.522 – 6.05 µg/kg lw), after exclusion of an outlier (1.540 µg/kg ww 28.25 µg/kg lw). Concentrations in Audouin's Gull eggs were statistically significantly different, with an average total Dechlorane Plus concentration of 0.029 µg/kg ww (or 0.398 µg/kg lw), and an overall range of 0.007 – 0.079 µg/kg ww (or 0.057 – 1.750 µg/kg lw).

Stable nitrogen ($\delta^{15}N$) and carbon ($\delta^{13}C$) isotope values were also determined in the samples. The relationship between the total concentration of Dechlorane Plus and the stable isotope values was tested simultaneously using a generalized linear model with the total concentration as the response variable and $\delta^{15}N$ and $\delta^{13}C$ as covariables. Pearson's correlation analysis was used to evaluate intraspecies relationships.

Based on the previous dietary studies in the area, both species are considered third-order consumers and no marked differences in their trophic position would be expected as both species feed mainly on fish on the Chafarinas Islands. However, Yellow-legged Gulls are known to be more opportunistic feeders than Audouin's Gulls, feeding more frequently on alternative food sources such as terrestrial prey (including Audouin's Gull eggs and chicks) and refuse tips. The stable isotope measurements in the current study confirmed this behaviour, with Yellow-legged Gulls showing significantly lower mean $\delta^{13}C$ (terrestrial prey and refuse tips generally show lower $\delta^{13}C$-values than marine prey) and higher mean $\delta^{15}N$-values than Audouin's Gulls. There was however considerable overlap in individual $\delta^{15}N$ values between the two species (range was 11.68-12.73‰ and 9.24–12.06‰ for Audouin's and Yellow-legged Gulls, respectively) suggesting that the relatively small difference in the mean $\delta^{15}N$ value between the two species (difference in the mean value was 1.32‰) was consistent with trophic positions of the two species being broadly similar. The generalised linear model analysis found that there was a significant relationship between $\delta^{13}C$ and the total Dechlorane Plus content in the gull eggs, with the total Dechlorane Plus concentrations decreasing similarly with increasing $\delta^{13}C$ within both species.

Overall it was concluded that the variations in concentration within and between the two species could be explained by foraging behaviour and diet rather than by trophic position. The relative increase in the consumption of terrestrial prey and/or food from refuse tips led to higher concentrations of Dechlorane Plus in the Yellow-legged Gulls.

The f_{anti} in the eggs was found to be similar in both species, with mean values of 0.81 for Yellow-legged Gulls and 0.82 for Audouin's Gulls. Anti-[DP-1Cl] was quantified in ~58 % of Yellow-legged Gull eggs but none of the Audouin's Gull eggs, suggesting that species-dependent factors influence the bioaccumulation and/or biotransformation of Dechlorane Plus. Anti-[DP-2Cl] was not detectable in any sample.
This study is not included in the registration dossier.

17. Zhang *et al.* (2010b) determined the levels of Dechlorane Plus in an aquatic food web consisting of invertebrates and fish from an electronic waste recycling site in Southern China. The samples include Hydrobidae (*Cipanopaludina catheyensis* and *Oncomelania* sp., n=5, TL=2.0), Crucian Carp (*Carassius auratus,* n=7, TL=2.7), Common Carp (*Cyprinus carpio*, n=6, TL=2.7), Walking Catfish (*Clarias batrachus*, n=6, TL=2.7) and Nothern Snakehead (*Channa argus*, n=7, TL=3.3). The samples analysed were either muscle samples (fish) or soft body parts (invertebrates). The limit of detection of the analytical method used was 0.01-0.05 µg/kg lipid. Dechlorane Plus was only detectable in a few samples of Hydrobidae (<LoD-14 µg/kg lipid weight) and Northern Snakehead (<LoD-12.5 µg/kg lipid) and the mean concentration in all species was <LoD. It is not possible to draw any meaningful conclusions from these data owing to the large number of non-detects.

    This study is not included in the registration dossier.

18. Kim *et al.* (2015) investigated the potential for biomagnification of Dechlorane Plus in seabirds from King George Island, Antarctica. The pectoral muscle tissue samples were collected between December 2008 and February 2009 from already dead animals. The species included Gentoo Penguin (*Pygoscelis papua*, n=2), Adélie Penguin (*Pygoscelis adeliae,* n=2), South Polar Skua (*Stercorarius maccormicki*, n=1) and Brown Skua (*Stercorarius antarcticus*, n=4). Dechlorane Plus was detected in all samples and the total concentrations were in the range 0.250-0.329 µg/kg lipid in the penguins and 2.12-11.1 µg/kg lipid in the skuas. The concentration of the anti-isomer was higher than that of the syn-isomer and the f_{anti} was between 0.71 and 0.78 in penguins and 0.69 and 0.89 in the skuas. Kim *et al.* (2015) estimated BMF values between skuas and penguins on the basis that skuas are predators of penguins, and these were reported to be 18.9 for syn-DP and 21.5 for anti-DP. These values indicate that biomagnification may be occurring from penguins to skuas. However these results should be treated with caution since (as noted by Kim *et al.*, 2015) both South Polar Skua and Brown Skua are migratory species, overwintering in low latitude regions of the Atlantic, South America and North Pacific, whereas penguins are known to spend their entire life in the Southern Ocean and Antarctica. In addition, the prey of skuas includes other birds, eggs and fish. Therefore there are significant uncertainties in these BMF values.

    This study is not included in the registration dossier.

19. The maternal transfer of syn- and anti-DP in Northern Snakehead (*Channa argus*) and Crucian Carp (*Carassius auratus*) has been studied by Wu *et al.* (2013). The two species are bottom-feeding species and were collected from an electronics waste recycling site in China. A total of 9 males, 9 females and 9 egg samples were collected for Nothern Snakehead and 5 males, 10 females and 10 egg samples were collected for Crucian Carp. Hepatic total Dechlorane Plus concentrations were between 250 and 1 920 µg/kg lipid in Nothern Snakehead and between 340 and 1 670 µg/kg lipid in Crucian Carp. In both species the levels in

males (median level 720 µg/kg lipid in Nothern Snakehead and 1 250 µg/kg lipid in Crucian Carp) were significantly ($p<0.05$) higher than in females (median level 580 µg/kg lipid in Nothern Snakehead and 540 µg/kg lipid in Crucian Carp). The total Dechlorane Plus concentration in eggs was between 4.6 and 310 µg/kg lipid indicating that maternal transfer was occurring. The mean egg/liver concentration ratios were 0.03 and 0.03 in Nothern Snakehead and 0.26 and 0.25 in Crucian Carp for syn- and anti-DP, respectively. A significant negative correlation between the egg/liver concentration ratio and the hepatic concentrations suggested that maternal transfer of Dechlorane Plus was occurring. Wu *et al.* (2013) also detected two dechlorinated analogues, anti-DP-1Cl and anti-DP-2Cl in liver and eggs, demonstrating maternal transfer of these analogues was occurring. The median f_{anti} was similar across both species and samples (0.58, 0.58 and 0.55 for Nothern Snakehead male liver, female liver and eggs, respectively; and 0.56, 0.55 and 0.55 for Crucian Carp male liver, female liver and eggs, respectively). A statistically significant correlation between the f_{anti} and the logarithm of the total Dechlorane Plus concentration in liver was found in the two species, which suggests that the concentration may influence the isomeric composition (i.e. concentration-dependent accumulation).

This study is not included in the registration dossier.

20. Li *et al.* (2014b) investigated the tissue distribution of syn-DP and anti-DP in wild frogs (*Rana limnocharis*) collected from an electronic waste recycling site in Southeast China. The frogs were collected from five sampling sites in July 2012 and pooled tissue samples of liver, muscle and brain from 53, 33, 33, 18 and 22 frogs from each site, respectively, were used for the analysis. In addition to syn-DP and anti-DP the study also considered the presence of two dechlorinated analogues (anti-DP-1Cl and anti-DP-2Cl).

    The limit of detection of the analytical method used was 0.096 µg/kg lipid for syn-DP, 0.19 µg/kg lipid for anti-DP and 0.049 µg/kg lipid for anti-DP-1Cl (no value was given for anti-DP-2Cl). Total Dechlorane Plus levels in the samples were in the range 2.01-291 µg/kg lipid. Anti-DP-2Cl was not detectable in any sample but anti-DP-1Cl was detectable in all liver and muscle samples and four out of five brain samples at concentrations up to 8.67 µg/kg lipid.

    The f_{anti} in liver, muscle and brain was 0.62, 0.82 and 0.72, respectively. There was no significant difference in the f_{anti} between muscle and brain but the f_{anti} was significantly ($p<0.05$) different between muscle and liver, indicating a preferential enrichment of the syn-isomer in liver. There was no significant difference in the concentrations in liver and muscle for syn-DP (the liver/muscle concentration ratio was close to 1, $p>0.03$) but there was a significantly higher concentration in muscle relative to liver for anti-DP (liver/muscle concentration ratio <1, $p<0.05$). The liver/brain concentration ratio for both isomers was above 1 ($p<0.05$), suggesting that the blood-brain barrier suppressed entry of the substances into brain tissue.

    This study is not included in the registration dossier.

21. Sühring *et al*. (2015) studied the distribution and maternal transfer of Dechlorane Plus in European Eels (*Anguilla anguilla*) during maturation. For the study, the contaminant distribution in artificially matured eels

was compared with that in muscle and gonads of yellow and silver eels[69] collected in a previous study. For the artificial maturation experiments a total of 16 female European Eels at the onset of spawning were collected from two German drainage systems and held in freshwater tanks for 7 days. Eleven individuals were used to determine the contaminant load prior to the onset of artificial maturation and the remaining five individuals were transferred to saltwater for 11 days and were then given weekly doses of salmon pituitary extract for up to 20 weeks in order to induce gonad maturation and ovulation. From week 16 onwards egg samples were also collected by biopsy in order to investigate oocyte maturation. Final oocyte maturation and ovulation was induced 48 hours after a sudden increase in body weight and body girth index had occurred and the eels were then sacrificed for analysis (one eel did not respond to this treatment). Muscle and gonad samples from 10 yellow and 10 silver eels (comparison group) from samples collected in 2012 for a previous study were also analysed.

Syn-DP, anti-DP and two dechlorinated products (anti-DP-1Cl and anti-DP-2Cl) were not detected in gonads of the yellow eels but showed increased concentrations in gonads and eggs of the hormone-treated eels compared with the comparison group. This suggests that the substances are not distributed to the gonads during the initial uptake processes but are transferred or re-destributed into gonads and eggs during the maturation process. However, it should be noted that there are no real negative controls in this study since the findings are based on comparison with other eels that had been exposed to Dechlorane Plus in the environment. The study found no maternal transfer into eggs of the 1,5-Dechlorane Plus mono-adduct DPMA.

This study is not included in the registration dossier.

22. Sun *et al.* (2015) investigated the bioaccumulation of Dechlorane Plus in biota from the Island Mangrove Nature Reserve, Pearl River estuary, South China. A total of 22 samples from four species were collected. The samples were muscle samples from Mud Crab (*Scylla serrata,* n=5), Chinese Black Sleepers (*Bostrichthys sinensis*, n=6), Red Eelgobys (*Odontamblyopus rubicundus*, n=6) and Daggertooth Pike Conger (*Maraenesox cinereus,* n=5). The concentrations of total Dechlorane Plus ranged between 0.18 and 6.88 µg/kg lipid and the highest concentrations were found in Daggertooth Pike Conger. Anti-DP was detectable in 100% of the samples and syn-DP was detectable in 54 % of the samples. The dechlorinated product anti-DP-1Cl was also detectable in 27.3 % samples at concentrations up to 2.65 µg/kg lipid. A significant positive correlation between the concentration of total Dechlorane Plus and the concentration of anti-DP-1Cl was found in the study.

The trophic level of each species was estimated based on $\delta^{15}N$ measurements assuming zooplankton represented trophic level 2 and using an enrichment factor of 3.8‰. This gave the trophic level as 3.95 for Mud Crab, 4.13 for Chinese Black Sleeper, 4.09 for Red Eelgobys and 5.03 for Daggertooth Pike Conger. The mean (and range) of total

[69] Yellow eels are mostly sedentary in their habitats (mostly rivers), where they build up high reserves of lipids in preparation for maturation into silver eels. Silver eels are mature eels that would naturally migrate back to their spawning grounds and have stopped feeding. They use up their stored lipids as energy reserves for the journey as well as development of gonads and eggs. The detection of a compound in muscle and gonads of yellow eels therefore indicates it is distributed throughout the different tissues during uptake rather than specifically as a result of maturation.

Dechlorane Plus determined in these species was 0.28 µg/kg lipid (0.18-0.38 µg/kg lipid), 1.13 µg/kg lipid (0.55-6.88 µg/kg lipid), 2.60 µg/kg lipid (1.75-4.31) µg/kg lipid and 2.76 µg/kg lipid (1.07-5.37 µg/kg lipid) respectively. Sun *et al.* (2015) determined the TMF for total Dechlorane Plus to be 2.31 for this food chain (p=0.07 indicating that the regression was not statistically significant). BMFs for various predator-prey combinations were also estimated. These were 11.8 for Daggertooth Pike Conger-Mud Crab, 2.92 for Daggertooth Pike Conger-Chinese Black Sleeper and 1.27 for Daggertooth Pike Conger-Red Eelgobys. These results suggest that biomagnification was occurring in this food web.

This study is not included in the registration dossier. The studies summarised above specifically consider bioaccumulation behaviour. Other biota monitoring data are reported in Appendix 3. N.B. This list is not necessarily exhaustive (see also Appendix 9).

3.4.2 Bioaccumulation in terrestrial organisms (soil-dwelling organisms, vertebrates)

No data on bioaccumulation in terrestrial organisms was included in the REACH registration dossier.

Although there are significant uncertainties in the physico-chemical data set, the combination of a predicted log K_{OW} >2 and log K_{OA} >5 indicate that Dechlorane Plus has the potential to biomagnify in terrestrial food webs if the rate of chemical transformation or metabolism is low, as suggested by Gobas *et al*. (2003) and Kelly *et al*. (2007). In particular, biomagnification factors of very hydrophobic substances (log K_{OW} >7) in terrestrial biota do not necessarily fall with increasing K_{OW}. It is therefore important not to overlook the potential for terrestrial bioaccumulation for Dechlorane Plus.

The findings from the literature search are summarized below.

3.4.2.1 *Laboratory studies*

- In a short-term toxicity study on the earthworm *Eisenia fetida*, Zhang *et al*. (2014) detected signs of oxidative stress and other damage following exposures up to 14 days (this study is summarised in more detail in Section 5.2). This indicates that the substance (or a more soluble impurity; the stated purity was >99 % w/w) can be taken up into earthworm tissues, but does not provide any quantitative information about bioaccumulation.

  Given the short duration of this study, it is of low relevance for this assessment. It is not useful to estimate an earthworm BCF using QSAR approaches given the uncertainty in the log K_{OW} value and limitations of the QSAR equations themselves.

- Chabot-Giguère *et al*. (2013) studied *in vitro* biotransformation of Dechlorane Plus using liver microsomes of breeding Ring-billed Gulls (*Larus delawarensis*) from Montreal, Canada. Adult male (n=19) and female (n=6) birds were randomly selected and live-captured during the incubation period. Shortly after capture, birds were euthanized and the left lobe of the liver was collected within 5 minutes. Liver microsomes were prepared by differential ultracentrifugation. Ethoxyresorufin-O-deethylase (EROD) activity measurement was used to verify the general hepatic microsomal CYP1A-like enzyme viability and to select individuals for the *in vitro* assays covering a gradient of EROD activities (i.e. low, medium and high activity).

A stock solution of Dechlorane Plus was prepared together with BDE-15 using isooctane, evaporated to dryness under nitrogen and reconstituted acetone to give a final concentration of 10 µg/mL for each isomer (and BDE-15). The *in vitro* assay was performed in triplicate using one adult male randomly selected from each of the three EROD activity groups to give a total of three individuals. Following addition of a phosphate buffer and 1 mg of the microsomal suspension to 3 µL of test solution made up to a final volume of 1 mL, test tubes were wrapped in aluminium foil and incubated at 41 °C (the physiological temperature of medium-size gulls) for 5 minutes before the sequential addition of a NADPH regenerating solution. The assay was allowed to proceed for 90 minutes in a 41 °C shaking (90 rpm) dry bath.

The syn- and anti- isomers were quantifiable in all nine liver samples, although the contribution of each isomer varied (this study used part of a larger data set reported by Gentes *et al*., 2012). None of the assays showed any depletion for either isomer over the 90-minute time frame of the assay (nor decabromodiphenyl ether in the same study). Degradation products were not screened for. In contrast, BDE-15 (included as a positive control) was significantly depleted (13, 31 and 6 % depletion in the low, medium and high EROD activity groups, respectively). Since NADPH was used as the sole cofactor in the assays, the results suggested that CYP isoenzyme-mediated reductive dehalogenation is unlikely to be a substantial metabolic pathway for Dechlorane Plus in this species, although the study authors pointed out that the time frame may have been insufficient to capture any depletion. In addition, the test concentration (30 µg/L) for each isomer significantly exceeds the solubility of the whole substance in pure water (ca. 2 ng/L). It is therefore possible that depletion could also have been impeded by the rate of dissolution, with any degradation masked by the high levels that were used.

This study is not included in the registration dossier.

- Peng *et al*. (2014) performed an *in vitro* experiment on metabolic transformation using liver microsomes isolated from Black-tailed Gull (*Larus crassirostris*). Dithiothreitol was included in the homogenization, wash, and resuspension buffers to preserve the catalytic activity of reductases. The final reaction volume contained either syn- or anti-Dechlorane Plus at a concentration of 50 ng/mL [µg/L]. The protein concentration in the reaction vial was 7.5 mg/mL and the CYP1A1-catalyzed EROD activity was 12 pmol/mg/min. Reactions were performed at 37 °C for 24 hours with constant agitation. Incubations without chemicals and without microsomes were used as negative controls to assess background contaminants and the possibility of non-enzyme mediated changes in chemical structure.

  No dechlorination of Dechlorane Plus was observed (no further details are provided). As for the study of Chabot-Giguère *et al*. (2013), the test concentration for each isomer significantly exceeds the solubility of the whole substance in pure water (around 2 ng/L) so it is possible that depletion could have been impeded by the rate of dissolution, with any degradation masked by the high levels that were used.

  This study is not included in the registration dossier.

- Zhang *et al*. (2015) investigated the bioaccumulation and translocation of Dechlorane Plus in rice (*Oryza sativa* L.). Three soils were used in the study: a control soil obtained from a rice production area in Hengli Town, Guangdong Province, a 'low exposure' soil consisting of paddy soils

obtained from close to e-waste recycling workshops and a 'high exposure' soil consisting of a mixture of e-waste contaminated soil (20%) and the control area soil (80%). Fertilizer was added to each soil in order to ensure that the nitrogen, phosphorus and potassium levels were consistent across all soils. The pH of the soils was between 6.2 and 6.9.

Rice seeds were sown on 10 July using the control soil and the seedlings were then transplanted into the two exposure group and control soils on 30 July. The plants were grown in a greenhouse for 40 days with daily watering. The pots did not contain drainage holes, in order to prevent loss. Each treatment group consisted of 30 pots, each containing 5 seedlings and 3 pots were pooled for analysis at the end of the experiment. The leaves, stems and roots were analysed separately. In addition, sections of some samples were used to assess plant growth and physiology.

The concentrations of Dechlorane Plus in the soils were determined both before and after planting and the mean concentration before planting was 0.24 µg/kg dw in the control soil, 36 µg/kg dw in the 'low exposure' soil and 220 µg/kg dw in the 'high exposure' soil. The mean concentration after planting was 0.16 µg/kg dw in the control soil, 28 µg/kg dw in the 'low exposure' soil and 160 µg/kg dw in the 'high exposure' soil.

Growth of the plants was assessed based on number of tillers and root morphogenesis after 40 days' exposure. Significant changes in plant morphology and physiology were observed in the plants in the two exposure groups compared with the control group. Most plants in the higher exposure group were withered and yellow and tillers in the two treatment groups were significantly lower than the control group. However, the soils used all contained a number of other substances as well as Dechlorane Plus (e.g. PCBs, PBDEs and other flame retardants) and so any effects seen cannot be ascribed solely to Dechlorane Plus.

Dechlorane Plus was detectable in the root, stem and leaves of all plants, including those in the control soil. The concentrations followed the general order root>leaf>stem. Zhang *et al.* (2015) considered that the fact that the concentration was generally higher in leaves than stem suggests that atmospheric uptake of Dechlorane Plus may occur as well as translocation, although the difference in concentration between leaf and stem was small in some cases (e.g. for the 'high exposure' group the mean (and range) concentration in leaves was 4.3 (3.2-6.7) µg/kg dw compared with 4.2 (3.6-7.0) µg/kg dw) so this conclusion could be misleading.

The f_{anti} in the soils was 0.75 for the 'high exposure' group, 0.67 for the 'low exposure' group and 0.68 for the control group. The f_{anti} in plant roots was significantly lower ($p<0.05$) than found in the corresponding soil and was found to decrease with translocation to the stem and the leaves (the values are only shown graphically in the paper but were around 0.55-0.67 in plant leaves). This implies that syn-DP is more bioaccumulative and/or is translocated more readily than the anti-DP isomer. Zhang *et al.* (2015) indicated that the root bioaccumulation factor (estimated as the concentration in root divided by the concentration in sediment) was higher for syn-DP than anti-DP but the values are not given in the paper. The root-stem translocation factor was determined to be in the range 0.14-0.22 and was slightly higher for syn-DP than anti-DP. The stem-leaf translocation factor ranged from 0.96-1.58 (no information is given as to whether the factor was larger for syn-DP or anti-DP).

The significance of the findings of this study is unclear. The growth of the plants in the two treatment groups was clearly affected by the contaminants present in the soil. Such toxic effects may have significantly

altered the uptake and distribution of the contaminants compared with healthy plants.

This study is not included in the registration dossier.

3.4.2.2 *Field studies*

- Yu *et al*. (2013) investigated the biomagnification of Dechlorane Plus in two terrestrial food chains. Common Kestrel (*Falco tinnunculus*) (n=23), Little Owl (*Athene noctua*) (n=14) and Eagle Owl (*Bubo bubo*) (n=10) specimens were obtained from the Beijing Raptor Rescue Centre, China, between January 2005 and July 2007. The specimens were either picked up dead, died during rehabilitation, or euthanized because of serious injuries. Eurasian Tree Sparrow (*Passer montanus*) (n=40, from nine locations according to Yu *et al*., 2011) and Brown Rat (*Rattus norvegicus*) (n=8, from three locations according to Yu *et al*., 2011) were captured in the locations where the birds had been collected (the timing is not stated). A field prey delivery study indicated that sparrows were the primary prey items of the kestrels (accounting for 50-83 % of their diet). Rodents form the vast majority (>90%) of the Eagle Owl's diet. Although not mentioned in the paper, Little Owls include a significant proportion of invertebrates in their diet. Pectoral muscle (approximately 1.5 g in dry weight each) was excised for analysis from the birds according to Yu *et al*. (2011), but no information is given about the tissue used for rats. Analysis was by gas chromatography-electron capture negative ionization mass spectrometry (GC/ECNI-MS) in selected ion monitoring (SIM) mode. The method detection limit was 0.2±1.4 µg/kg lw.
  Median concentrations (and ranges) of Dechlorane Plus (both isomers) were as follows:

Common Kestrel:	1.5 (<LoD – 60) µg/kg lw
"Owl"[70]:	49 (7.0 – 500) µg/kg lw
Eurasian Tree Sparrow:	4.9 (<LoD – 31) µg/kg lw
Brown Rat:	24 (<LoD – 160) µg/kg lw

  Based on mean lipid weights of 12.2 %, 8.9 %, 10.4 % and 20.6 % for each of these species, the median concentrations would be approximately 0.18, 4.36, 0.51 and 4.94 µg/kg ww for Common Kestrel, "Owl", Eurasian Tree Sparrow and Brown Rat, respectively. No stereoselection was apparent in the pattern of bioaccumulation in any species.

  Anti-DP-1Cl was detected in approximately 30% of the samples, with a strong positive correlation (Pearson's correlation, $r^2 = 0.68$, $p < 0.01$) between this substance and anti-Dechlorane Plus. Average ratios of anti-DP-1Cl to anti-Dechlorane Plus were 0.038 ± 0.024, 0.048 ± 0.035, and 0.011 ± 0.012 for the kestrels, owls and sparrows, respectively.[71]

  BMFs were calculated as the ratio between the median lipid normalized concentrations in the predator and prey. The calculated BMF was 2 in the rat–owl food chain, indicating biomagnification, and 0.3 for the sparrow–kestrel

[70] No significant differences (*t*-test, $p > 0.05$) in concentrations were observed between the two owl species, and therefore their data were combined and treated as one population.

[71] The study authors suggest that this indicates biotransformation of DP and/or accumulation of anti-DP-1Cl along with anti-DP from the environmental matrix. They also suggest that kestrels may have a greater metabolic capacity than sparrows since the DP-1Cl/DP ratio is higher in the former species. This is speculative given the limited data available from this study so it is not discussed further.

food chain, indicating biodilution[72]. (When wet weight is considered, the BMFs become 0.9 and 0.4, respectively.) No significant differences were found for the BMF values of the two isomers in either feeding relationship.

The use of muscle tissue for the birds might mis-represent the total whole body concentrations, which could affect the conclusions (especially if lipid weight concentrations were different between tissues). It is not clear how the calculations would be affected if the concentrations were expressed as a geometric mean rather than median concentration. The representivity of the samples is also unknown. For example, median total concentrations in six Common Kestrels collected in Beijing in 2004 to 2006 by Chen *et al*. (2013b) were reported as 550 µg/kg lw (liver) and 810 µg/kg lw (muscle) (see Appendix 4**Error! Reference source not found.**). These are two orders of agnitude higher than reported in the study of Yu *et al*. (2013), and so the corresponding BMF would be above unity for the sparrow-kestrel food chain if these data were used. (Conversely, if the concentration in sparrows is usually lower than reported in this study, the BMF would reduce.)

This study is not included in the registration dossier.

- Barón *et al*. (2014a) analysed sterile eggs (n=115) of fourteen different bird species from Doñana Natural Park and surrounding areas, in south-west Spain. The samples were collected opportunistically during three campaigns in 2010, 2011 and 2012. All the eggs were frozen and sent to the laboratory in individual protected containers, where they were measured, broken and the contents weighed, homogenized and freeze dried and stored at −20 °C until analysis.
  Samples were spiked with ^{13}C-syn-DP and then extracted before analysis using a GC system coupled to a GC/MS triple quadrupole, working in negative chemical ionization mode using methane as reagent gas. To enhance the sensitivity and selectivity, selective reaction monitoring mode was applied. The most intense transition was used for the quantification and the second transition was used for confirmation. Transitions monitored were 654 > 35 and 654 > 37 for syn- and anti-DP, and 664 > 35 and 664 > 37 for ^{13}C-syn-DP.

  Recoveries were evaluated by spiking purchased eggs with 5 ng of each compound. Four replicates were made and the compounds found in the blank samples did not exceed 5 % of the amount spiked in any case. The method showed good recoveries for halogenated norbornenes (73 to 89 %). The identification and confirmation of halogenated norbornenes were based on the following criteria: (i) simultaneous responses to the two selected ions or transitions were needed, (ii) the area of signal must be at least three times higher than the signal noise, and (iii) the difference in the relative intensity of a peak respect to theoretical values obtained with standard solutions cannot exceed ±15 %. To prevent interferences and contamination several procedural blanks were made. The contribution of the blank to the signal never exceeded 5 %. Method detection limits (MDLs) were determined as the minimum amount of analyte producing a peak with a signal-to-noise ratio of 3, and the method quantification limit (MQL) was determined as the minimum amount of analyte which produces a peak with a signal-to-noise ratio of 10. MDLs for dechloranes ranged from 0.01 to 0.9 ng/g lw and MQLs ranged from 0.03 to 3.0 ng/g lw. Dechlorane Plus was detected in all fourteen species' eggs (see Table 10).

  The highest levels of total isomers were found in White Stork, with a mean value of 24.1 µg/kg lw, and one individual egg containing 102 µg/kg lw (0.1

[72] For comparison, BMFs of 1.3 to 6.9 were determined for hexa- and octaBDE congeners in the sparrow–kestrel food chain, and higher BMFs were obtained when prey items from lower trophic levels (e.g. grasshoppers) were considered (Yu *et al*., 2011). That study did not report levels in owls.

mg/kg lw). The paper does not discuss this specifically, but this species is known to eat a wide variety of aquatic and terrestrial organisms, and may scavenge at rubbish dumps on migration and at the wintering quarters. Species with more aquatic diets (i.e. Glossy Ibis, Purple Heron, Slender-billed Gull, Gull-billed Tern and Gadwall) tend to have lower egg concentrations than those with more terrestrial diets (e.g. kites and eagle), although this was not always the case (e.g. Common Kestrel eggs had slightly lower levels than those of the ibis and heron).The mean f_{anti} values (0.57 – 0.76) were similar to those found in commercial mixtures, suggesting that isomer specific accumulation of the syn- isomer does not occur in birds.

Table 10: Dechlorane Plus concentrations in bird egg samples from Spain (from Barón *et al*., 2014a)

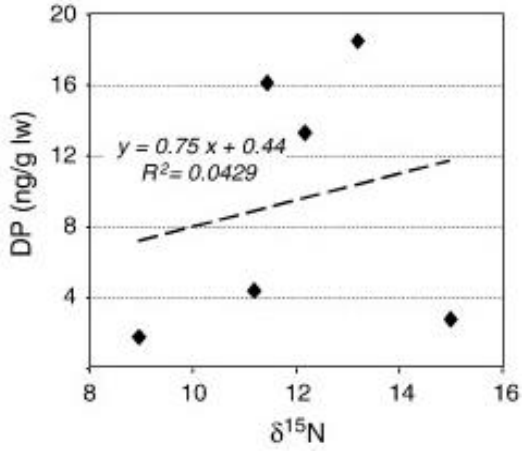
Species	n	Mean Dechlorane Plus concentration, µg/kg lw (with associated standard deviation; and range)		
		syn-	anti-	Total
Falconiformes				
Black Kite *Milvus migrans*	22	5.19 (3.42; NQ–10.6)	10.9 (7.24; NQ–24.3)	16.1 (10.4; NQ–34.4)
Red Kite *Milvus*	2	4.50 (1.48; 3.46–5.55	14.0 (3.49; 11.5–16.5)	18.5 (4.97; 15.0–22.0)
Western Marsh Harrier *Circus aeruginosus*	1	0.85 (–)	1.91 (–)	2.76 (–)
Booted Eagle *Aquila pennata*	6	4.43 (2.99; 0.83–8.05)	8.89 (5.12; 2.82–14.5)	13.3 (7.73; 3.65–22.0)
Common Kestrel *Falco tinnunculus*	13	2.45 (4.02; ND–12.3)	2.15 (1.78; ND–4.72)	4.33 (5.21; ND–17.0)
Black-winged Kite *Elanus caeruleus*	1	0.63 (–)	1.08 (–)	1.72 (–)
Ciconiiformes				
Glossy Ibis *Plegadis falcinellus*	4	2.51 (1.30; 1.00–3.99)	3.96 (1.80; 1.99–4.81)	6.48 (3.10; 2.99–10.0)
Purple Heron *Ardea purpurea*	3	2.84 (2.40; 0.23–4.95)	3.86 (2.98; 0.54–6.31)	6.71 (5.38; 0.77–11.3)
White Stork *Ciconia ciconia*	34	8.76 (9.14; ND–45.3)	15.8 (14.6; ND–73.4)	24.1 (22.0; ND–102)
Strigiformes				
Barn Owl *Tyto alba*	1	3.07 (–)	2.38 (–)	5.44 (–)
Charadriiformes				
Slender-billed Gull *Chroicocephalus genei*	3	0.41 (0.37; 0.12–0.83)	0.47 (0.27; 0.17–0.71)	0.87 (0.63; 0.28–1.53)
Black-headed Gull *Chroicocephalus ridibundus*	7	1.30 (1.26; 0.24–3.93)	3.10 (3.74; 0.55–11.3)	4.40 (4.99; 0.79–15.2)
Gull-billed Tern *Gelochelidon nilotica*	8	1.17 (1.07; 0.16–3.38)	1.64 (0.61; 0.96–2.56)	2.81 (1.51; 1.25–5.70)
Anseriformes				
Gadwall	10	0.94	1.99	2.93

Species	n	Mean Dechlorane Plus concentration, µg/kg lw (with associated standard deviation; and range)		
		syn-	anti-	Total
Anas strepera		(0.20; 0.61–1.20)	(0.92; 1.20–3.75)	(0.94; 2.00–4.93)

Note: n: Sample number, ND: Not detected, NQ: Not quantified

Stable isotope analysis was used to investigate biomagnification potential. High $\delta^{15}N$ values were observed for Gadwall, possibly due to the high levels of anthropogenic nitrogen in the aquatic ecosystem (water flowing into the Doñana National Park is susceptible of nitrate contamination from surrounding urban areas and strawberry/rice farming). The $\delta^{13}C$ values indicated significant differences in dietary carbon sources between species, but similar values were determined for the Falconiformes (between −21.40 and −26.32). This last group was used to assess biomagnification processes along the trophic chain, as they had dissimilar $\delta^{15}N$ values (between 8.95 and 14.98) (see Figure 6, the dotted line represents the linear regression).

Figure 10: Plot of mean total Dechlorane Plus concentrations in falconiform eggs versus $\delta^{15}N$



(reprinted with permission from Barón *et al*. (2014a). Copyright 2014: Environment International)

The analysis presented in the paper does not consider the accumulation of the individual isomers, but total Dechlorane Plus levels (dominated by the anti-isomer) were not clearly linearly correlated with trophic position ($r^2 = 0.04$) (unlike PBDEs in the same samples). However, since only one egg sample was available for two of the species (Western Marsh Harrier and Black-winged Kite), one of which occupied the highest trophic position according to $\delta^{15}N$ value, these results should be interpreted with caution. For example, if the highest data point were removed (or the concentration were higher), the linear trend would be more strongly positive.

All of the sampled Falconiform species are apex predators, with diets comprising other (mainly terrestrial) organisms for which concentration data are not available. It would therefore be premature to conclude that there is no biomagnification in specific feeding relationships. In addition, the use of eggs to indicate biomagnification potential might not be reliable if there are differences in transfer efficiency between mothers and eggs in different species

(i.e. the concentrations in adult birds might be higher than suggested by the egg data, as demonstrated for Domestic Chickens by Zheng *et al*., 2014a). Many of the species are migratory, and given the potential for accumulation over the long-term, their body burdens might not necessarily reflect their diet in a particular area. This study therefore does not provide strong evidence of biomagnification as such, but it clearly shows that Dechlorane Plus is a contaminant in a variety of European bird species using different habitats and food sources (in a protected environment without obvious point sources) and that transfer to eggs (a sensitive life stage) can occur at levels up to 0.1 mg/kg lw in wild birds.

This study is not included in the registration dossier.

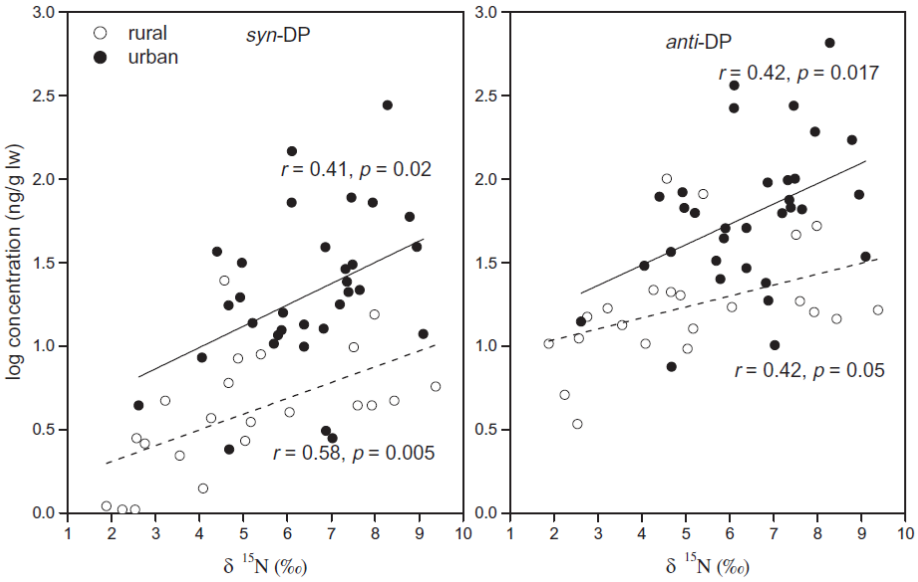
- Sun *et al*. (2012) investigated the relationship between Dechlorane Plus levels and trophic position in tissues from three terrestrial passerine bird species. Light-vented Bulbul (*Pycnonotus sinensis*, n=16), Long-tailed Shrike (*Lanius schach*, n=19), and Oriental Magpie-robin (*Copsychus saularis*, n=19) were collected from seven sites in the Pearl River Delta region of southern China between September 2009 and May 2010. Four of the sites had highly developed industries and were considered urban locations. The other three sites were characterized by agricultural activities so were considered rural locations. The three species are resident with relatively small territories and foraging areas. Captured birds were transported immediately to the laboratory, euthanized and then pectoral muscle and liver were excised from each bird and stored at -20 ºC until chemical analysis.
  Following extraction, analysis was performed by GC/MS using electron capture negative ionization in the selective ion monitoring mode. Procedural blanks were performed in each batch of samples. Trace amounts of syn- and anti- isomers were detected in procedural blanks (n=10) with mean concentrations of 0.43 and 1.14 ng/mL (accounting for 5.3 % and 8.5 % of the sample with the lowest Dechlorane Plus level), respectively, and these were subtracted from the sample results. Recoveries of syn- and anti- isomers were evaluated by spiking known concentrations of the target isomers in solutions and matrix. The mean recoveries of syn- and anti- isomers in three spiked blanks were 87 ± 5.2 % and 92 ± 7.8 %, and 96 ± 8.5 % and 91 ± 10.2 % in three matrix spikes, respectively. The mean recovery of the surrogate standard (^{13}C-PCB-141) was 101 ± 14.3 % so reported concentrations were not corrected for surrogate recovery. The LoQ was 0.35, 2.28 and 0.03 µg/kg for syn-DP, anti-DP and anti-DP-1Cl/anti-DP-2Cl, respectively. Concentrations below the LoQs were considered to be zero in the subsequent data analysis.

  Both Dechlorane Plus isomers were detected in all samples. The concentration range (total isomers) was 3.9 – 930 µg/kg lw in muscle and 7.0 – 1 300 µg/kg lw in liver, respectively. Birds from urban sites had significantly higher concentrations than from rural sites (mean: 300 versus 73 µg/kg lw, $p < 0.01$), suggesting that contamination is linked to industrialization/urbanization.

  All (except three bulbul and four shrike) samples had ratios of liver concentration to muscle plus liver concentration statistically significantly higher than 0.5 (for both isomers) ($p < 0.05$), suggesting liver-specific accumulation. However, since muscle has a larger biomass than the liver, muscle concentrations were used for inter-species comparisons. ANOVA applied to samples from rural and urban sites separately indicated significant differences in Dechlorane Plus concentrations among bird species at both rural and urban sites ($p < 0.05$). Concentrations increased in the order of bulbul < shrike < magpie-robin, with concentrations in bulbul being significantly lower than the other two species ($p < 0.05$).

Bulbuls are polyphagous and eat primarily plant matter, whereas the other two species eat insects and small vertebrates. Average $\delta^{13}C$ values for the bulbul were significantly lower than those of the other two species, confirming that the dietary carbon sources were different. Stable nitrogen isotope ratio ($\delta^{15}N$) values in muscle varied widely for each species, i.e. 1.88 – 9.10‰ for bulbul, 2.57 – 8.79‰ for shrike, and 3.22 – 9.38‰ for magpie-robin, respectively. Linear regression was therefore performed using data from individual samples rather than at the species level. A positive correlation was found between log normalized concentrations of Dechlorane Plus (syn- and anti- isomer separately) and $\delta^{15}N$ values at both rural and urban sites (see Figure 7). Whilst this could imply biomagnification, comparisons of trophic position between locations based on raw $\delta^{15}N$ data are inappropriate since the baseline $\delta^{15}N$ values for each site are unknown.

Figure 11: Plot of mean Dechlorane Plus concentrations in passerine muscle versus $\delta^{15}N$



(reprinted with permission from Sun *et al*. (2012). Copyright 2012: Elsevier Ltd)

No significant difference in the f_{anti} values between muscle and liver was observed so an overall f_{anti} was calculated for the three species separated into two groups (urban and rural). The f_{anti} values in birds from rural sites were significantly higher than those from urban sites ($p < 0.05$). In addition, there was a significant negative correlation between the f_{anti} values and $\delta^{15}N$ at both rural and urban sites ($p < 0.05$). This result suggests that higher trophic organisms may preferentially accumulate the syn- isomer.

No anti-DP-2Cl was detected in any sample, but anti-DP-1Cl was detected at up to 7.6 µg/kg lw at frequencies of 28, 37 and 55 % in bulbul, shrike and magpie-robin, respectively. Another substance found in 15 of the samples (up to around 41 µg/kg lw) was believed to be syn-DP-1Cl, but this could not be confirmed due to the lack of an authentic standard.

The use of lipid normalized concentrations in muscle to indicate biomagnification potential might not be reliable if they are not representative of whole body concentrations. It is not clear how the calculations would be affected if the concentrations were expressed as a geometric mean rather than median concentration, or wet weight concentrations. The variation in trophic

status is also surprisingly wide for each species. As noted above, plotting all raw $\delta^{15}N$ data together from different sites is not appropriate. It is unfortunate that there are no data on levels in food items, so the actual level of accumulation from the diet cannot be assessed. This study therefore only provides equivocal evidence that contamination levels increase with trophic position in terrestrial passerines, although it shows that levels may reach up to around 1 mg/kg lw in some tissues in wild bird species.

This study is included in the registration dossier as supporting monitoring information and is considered fully reliable by the Registrant. Although the bioaccumulation aspects are not discussed in the CSR, the study summary does mention that trophic level is an important factor for levels and isomeric composition in birds.

- Yu *et al*. (2014) investigated the relationship between Dechlorane Plus levels and trophic position in tissues from two terrestrial passerine bird species. Eurasian Tree Sparrows (*Passer montanus*, n=67) were collected outside the breeding season from various sites within two major cities (Beijing (n=40) and Guangzhou (n=19)) and a rural reference site (Shaoguan, Guangdong Province (n=8)) in China between August 2009 and May 2011. In addition, Common Magpies (*Pica pica*, n=9) were collected in the same period from one site in the suburbs of Wuhan, China. Captured birds were transported immediately to the laboratory, euthanized and then pectoral muscle was excised from each bird and stored at -20 ºC until chemical analysis.
  Following extraction, analysis was performed by GC/MS using electron capture negative ionization in the selective ion monitoring mode. Procedural blanks were consistently analyzed and the mean values were subtracted from the sample results (Dechlorane Plus isomers were found at levels <10% of that in the samples). Matrix spiking triplicates and surrogate standard recoveries from authentic samples were also used for quality control purposes. The limit of detection was in the range 0.14-0.17 µg/kg lw for the two isomers.

  Dechlorane Plus (total isomers) was found in all magpie muscle samples from Wuhan (median: 4.4, range: 1.7-18 µg/kg lw), and was detected in sparrow muscle samples at concentrations up to 350 µg/kg lw. Levels in Guangzhou (median: 13, range: not detected-350 µg/kg lw) were significantly higher than those in Beijing (median: 4.9, range: not detected-31 µg/kg lw)[73] ($p < 0.01$), which was believed to reflect the intensive e-waste recycling activities in the southern region (although no e-waste recycling activities occurred in the sampling locations). In contrast, no statistically significantly difference was found between levels in Guangzhou and the reference site (median: 24, range: 8.9-120 µg/kg lw), whereas the levels of other flame retardants were much lower at the latter site compared to the cities.

  Sparrows eat predominantly seed and grain, whereas magpies are omnivorous. Samples from Beijing and Guangzhou exhibited a wider range of $\delta^{13}C$ and $\delta^{15}N$ values compared to those from the other two locations, indicating a high heterogeneity of diet source for sparrows in the two former cites. In general, mean $\delta^{13}C$ values in samples from Beijing (18.7±3.3‰) and Guangzhou (-20.2±4.7‰) were significantly higher than those in samples from the reference site (-24.7±1.1‰) and Wuhan (-23.8±0.4‰) ($p < 0.005$). In contrast, the mean $\delta^{15}N$ values of samples from the reference site (8.7±0.4‰) were higher than those from the cities (Beijing: 6.8±1.3‰, Guangzhou: 6.0±1.4‰, Wuhan: 5.5±1.2‰)) ($p < 0.001$). This may partly be explained

[73] These data appear to be the same as reported in Yu *et al*. (2013).

by the different isotopic baselines as well as the variation in isotopic fraction among different species and ecosystems.

Comparisons of trophic position based on raw $\delta^{15}N$ data are inappropriate since the baseline $\delta^{15}N$ values for each ecosystem are unknown. Unfortunately, the paper does not discuss any linear correlation between the levels in the birds and their trophic position at specific localities using data from individual samples. Data for individual samples are not provided in the supporting information.

The use of muscle and lipid normalized concentrations to indicate bioaccumulation potential might not be reliable if they are not representative of whole body concentrations. It is not clear how the calculations would be affected if the concentrations were expressed as a geometric mean rather than median concentration. It is unfortunate that there are no data on levels in food items, so the actual level of accumulation from the diet cannot be assessed. The variation in $\delta^{15}N$ values for the same species at different sites also confounds the interpretation of the results. No conclusion is drawn about biomagnification in terrestrial passerines from this study for the purposes of this assessment, but it provides clear evidence of widespread contamination, with levels up to 0.35 mg/kg lw in a granivorous species.

This study is not included in the registration dossier.

- Peng *et al.* (2015) investigated the accumulation of Dechlorane Plus in terrestrial passerines. The birds were collected from a national nature reserve in South China around 120 km from a major electronic waste recycling area. A total of 44 muscle samples from the following species were analysed: Great Tit (*Parus major,* n=18), Oriental Magpie-robin (*Copsychus saularis*, n=7), Red-whiskered Bulbul (*Pycnonotus jocosus*, n=6), Light-vented Bulbul (*Pycnonotus sinensis,* n=5), Streak-breasted Scimitar Babbler (*Pomatorhinus rufficollis*, n=4), Long-tailed Shrike (*Lanius Schach*, n=2) and Orange-headed Thrush (*Zoothera citrina*, n=2). The levels of total Dechlorane Plus ranged from 1.2 to 104 µg/kg lipid and the levels were found to be significantly (p=0.03) higher in insectivorous birds (mean 16.9 µg/kg lipid; n-=27) than in omnivorous birds (mean 6.4 µg/kg lipid; n-17). As insectivorous birds generally occupy a higher trophic level than omnivorous birds Peng *et al*. (2015) considered that this could be an indication of biomagnification. Unfortunately the trophic levels of the birds in the study were not determined. The f_{anti} in the samples ranged from 0.34 to 0.97, with the median f_{anti} for individual species in the range 0.55-0.82, which was considered by Peng *et al*. (2015) to be similar to that in technical Dechlorane Plus products (0.64-0.80). There was no significant difference in the f_{anti} between omnivorous birds and insectivorous birds, but interspecies differences were evident (for example both the Red-whiskered Bulbul and Oriental Magpie-robin had a significantly higher f_{anti} than the Great Tit and Light-vented Bulbul). It could not be established if these difference resulted from different isomeric profiles of Dechlorane Plus in the diets or from different metabolic potential.
  This study is not included in the registration dossier.
- Muir *et al.* (2014) [ABST] analysed a food web in a remote area of the Canadian Arctic (western Nunavut, Bathurst region). This included vegetation (e.g. various lichen, moss, willow, graminoid species, etc.), Caribou (*Rangifer tarandus groenlandicus*) and Grey Wolf (*Canis lupus*). Using estimated total body burdens, the TMF for the anti- isomer was below unity (0.83), suggesting that trophic magnification was not occurring. A Caribou-Wolf BMF of 3.4 ± 2.1 was estimated for the anti- isomer, but this was not statistically significant. No information is provided for the syn- isomer and no other details are available

(e.g. it is not known how many animals were involved, which tissues were sampled or how body burden was estimated for an animal as large as a Caribou). The reliability of this study is therefore unknown.

This study is not included in the registration dossier.

Other terrestrial wildlife monitoring results from the open literature are reported in Appendix 4. In addition, a number of studies have examined contamination of human tissues and these are briefly summarised below.

- Ren *et al*. (2009) collected serum samples from residents at two sites in southern China during 2005. The first cohort consisted of 20 residents from an electronic waste (e-waste) dismantling site in Guiyu town, Shantou City, Guangdong Province (where 80% of families are engaged in primitive recycling work). The second cohort consisted of 20 residents of Haojiang district, Shantou City, about 50 km east of Guiyu, where fishing was the predominant industry. Dechlorane Plus was detected in all the samples, with concentrations (total isomers) ranging from 7.8 to 465 (median: 42.6) µg/kg lipid weight (lw) in Guiyu and 0.93 to 50.5 (median: 13.7) µg/kg lw in Haojiang. Levels were broadly similar to (though slightly lower than) those of decabromodiphenyl ether in the same samples. The average f_{anti} was 0.58±0.11 and 0.64±0.05 for Guiyu and Haojiang, respectively ($p < 0.01$). A substance tentatively identified as DP-1Cl was found in 11/20 samples from Guiyu and 8/20 samples from Haojiang. No hydroxylated metabolites were detected. Dechlorane Plus isomers were detected in a pooled serum sample from a reference population from Guangzhou (a typical urban city in south China), but the concentration was below the limit of quantitation.

  The volunteers' ages ranged from 23 to 67 years, and 73 % were male (27 % female). Serum concentrations were not correlated with age in either the Guiyu or Haojiang samples. According to the results of the questionnaire, most of the dismantling workers in Guiyu were temporary migrants from other parts of China and had worked there for various lengths of time. Food consumption is not thought to be the main route of human exposure in Guiyu, because most food is bought in from other regions. Thus, inhalation of Dechlorane Plus from the air and from dust is likely to be more important.

  This study is included in the registration dossier as supporting monitoring information and is considered fully reliable by the Registrant.

- Yan *et al*. (2012) measured Dechlorane Plus in human blood samples collected between May and July 2011 from 70 occupationally exposed workers from e-waste recycling workshops in Longtang Town, Qingyuan County, south China, and 13 residents of Guangzhou City (50 km distant) with no occupational exposure acting as a control group. The volunteers were between 20 and 59 years old, and the male to female sex ratio was 48:52.

  Anti-Dechlorane Plus was consistently detected in all serum samples. The syn- isomer was detected in all samples from e-waste recycling workers but in only 3 out of 13 (23 %) samples from the control group (which might be partly attributed to its relatively high LoQ of 3.08 µg/kg lw, as well as the lower levels in urban residents). Concentrations of total isomers in serum ranged from 22 to 2 200 µg/kg lw (median: 150 µg/kg lw) in occupationally exposed workers and from 2.7 to 91 µg/kg lw (median: 4.6 µg/kg lw) in the control group. No significant correlation was observed between serum concentrations and age or occupational exposure time

when the whole sample set was considered. However, Dechlorane Plus concentrations were significantly correlated with age in women, which was thought to possibly reflect differences in selective absorption and elimination and/or gender-specific metabolic half-life. The maximum concentration (2 200 µg/kg lw) was detected in a 48 year old woman who worked in e-waste dismantling.

Anti-DP-2Cl was not detected in any sample (LoQ 0.51 µg/kg lw). Anti-DP-1Cl was detected in 51 of the 70 samples (73 %) from occupationally exposed workers at concentrations up to 9.93 µg/kg lw (median: 1.47 µg/kg lw), but not in any of the control group samples (LoQ 0.64 µg/kg lw). The higher f_{anti} values and lower ratios of anti-DP-1Cl to anti-DP in women than men suggested that women might have a lower metabolic capacity for Dechlorane Plus than men, and hints at the possibility that dechlorination might occur *in vivo*.

This study is not included in the registration dossier.

- Ben *et al*. (2014) measured Dechlorane Plus in matched human maternal blood−placenta−cord blood samples collected between July 2010 and July 2011 from 72 residents of the e-waste recycling area of Wenling, Taizhou region, China. All donors were required to be residing in a rural area. The R20 group (n=48) contained donors who had lived in Wenling for more than 20 years and were living or had recently lived in villages where e-waste recycling activities were undertaken but did not directly participate in e-waste recycling activities. The R3 group (n=24) contained donors who had lived in Wenling for less than 3 years, had not previously lived in villages where e-waste recycling activities were undertaken, and did not participate in e-waste recycling activities.

  Both isomers of Dechlorane Plus were detected in all of the samples, with anti- isomer levels approximately double those of the syn- isomer. Maternal serum concentrations were highest, and were two to three times higher in the R20 group (geometric mean of 13.5 µg/kg lw, range 1.28−900 µg/kg lw) than those in the R3 group (a statistically significant difference). Total concentrations in most of the cord sera samples were 1.35−4.76 µg/kg lw (first to third quartiles) with a small number of samples having concentrations up to 89.7 µg/kg lw, indicating that individual foetuses had widely different body burdens. A similar skewed concentration distribution was found in the placentas and maternal sera. The concentration ratio in the cord and maternal serum was estimated to be 0.45 for the syn- isomer and 0.35 for the anti- isomer, suggesting that the placenta partially limited transfer for the anti- isomer. The anti- isomer/total Dechlorane Plus concentration ratios in the placentas and cord sera were significantly different from those in the maternal sera, suggesting stereoselection.

  Anti-[DP-2Cl] was not detected in any of the samples. Several possible monodechlorinated analogues of Dechlorane Plus were found, but with the exception of anti-[DP-1Cl] the peaks could not be quantified in the absence of authentic standards. For maternal sera, placenta, and cord sera, anti-[DP-1Cl] was detected in 81 %, 69 %, and 36 % in the R20 group and 71 %, 29 %, and 4 % in the R3 group, respectively. The concentration of anti-[DP-1Cl] ranged from not detected (0.029 or 0.064 µg/kg lw) to 17.2 µg/kg lw.

  This study is not included in the registration dossier.

- In a related study, Ben *et al*. (2013) measured Dechlorane Plus in blood serum and breast milk from women living at e-waste recycling sites in

Wenling, Taizhou region, China. Forty-four breast milk samples and forty-five serum samples (including 40 breast milk and serum samples from the same women) were collected from July 2010 to March 2011 (n = 49 in total). Both syn- and anti- isomers were detected in all samples. Levels in people who had been resident in the local environment for more than 20 years (R20 group) were significantly higher than those living there for less than three 3 years (R3 group) ($p < 0.05$). For the R20 group, arithmetic means in serum were 25.4 (range: 0.56 – 278, median: 2.79) and 46.1 (range: 1.24 – 656, median: 5.95) µg/kg lw for the syn- and anti-isomers, respectively; arithmetic means in breast milk were 10.4 (range: 0.29 – 139.2, median: 1.33) and 27.4 (range: 0.71 – 451, median: 3.32) µg/kg lw for the syn- and anti- isomers, respectively. The arithmetic mean levels of syn-, anti- and total isomers in the R20 group were much higher than the median levels, demonstrating a skewed distribution. The highest total isomer concentration was 900 µg/kg lw in blood serum, so the data set for this matrix appears to be the same as that reported in Ben *et al*. (2014).

The milk/serum partition coefficient from the same women was approximately 0.43 and 0.47 for the syn- and anti- isomer, respectively. Similar f_{anti} values in serum and milk suggested that stereoselective bioaccumulation did not occur during transfer from blood to milk.

DP-1C1 was detected in 84 % of serum samples and 45 % of milk samples (maximum concentrations were 14.9 and 7.19 µg/kg lw in blood and milk, respectively). DP-2Cl was not detected in any sample.

This study is included in the registration dossier as supporting monitoring information and is considered fully reliable by the Registrant.

- Zhang *et al*. (2013) measured Dechlorane Plus concentrations in two groups of workers at a manufacturing site in eastern China and a non-occupationally exposed control group residing 3 km from the plant. It was detected in all whole blood samples (n = 47), with total concentrations in the range 89.8 – 2 958 µg/kg lw (median: 456 µg/kg lw). The control group (n=12) had the lowest concentrations, in the range 89.8 – 513 µg/kg lw (mean 243 µg/kg lw). Dechlorane Plus was detected in all human hair samples (n=43), with total concentrations in the range 4.08 – 2 159 µg/kg dw.[74] As with blood, the control group had the lowest concentrations (4.08 – 236 µg/kg dw with an exception of 356 µg/kg dw). Significantly positive correlations (r^2: 0.60−0.76) were found for the logarithmic concentrations of syn-, anti-, and total isomers in human blood against working years in the group associated with the manufacturing process.

  This study is not included in the registration dossier.

- He *et al*. (2013) and Wang *et al*. (2014) analyzed pooled human blood serum samples from different age groups from the south coast of Laizhou Bay, Shandong Province, China. The mean concentration of Dechlorane Plus (total isomers) was 3.6 µg/kg lw (range: 1.4–11 µg/kg lw). No trend was found between concentration and age, although the younger

[74] Zheng *et al*. (2010) also detected Dechlorane Plus in human hair samples collected from an e-waste recycling area and two control areas (rural and urban) in south China, at concentrations ranging from 0.02 to 58.32 µg/kg. Anti-DP-1Cl was also detected in human hair (mainly from the e-waste recycling area), with concentrations up to 0.23 µg/kg; correlations with the levels of Dechlorane Plus in hair and dust suggested that it was present in the environmental matrix rather than formed *in situ*.

volunteers (in the 20–29 year-old group) had the highest serum concentrations. The authors noted that Dechlorane Plus was first produced in China in 2003. Inhalation exposure was thought to be important.

This study is not included in the registration dossier.

- Chen *et al*. (2015) collected 34 matched human hair and serum samples (19 males and 15 females) from e-waste recycling workers in South China. Dechlorane Plus (total isomer) concentrations ranged from 6.3 to 1 100 µg/kg dw in hair and from 22 to 1 400 µg/kg lw in serum. Levels of anti-DP-1Cl ranged from 0.02 to 1.8 µg/kg dw in hair and from not detected to 7.9 µg/kg lw in serum. A significant difference was found in the Dechlorane Plus isomer composition between hair and serum, suggesting stereoselective bioaccumulation. A sharp gender difference was found in the levels of Dechlorane Plus in hair. Moreover, both Dechlorane Plus isomers and anti-DP-1Cl in hair significantly correlated with those in serum for male samples, but not for female samples. The observed gender differences may be partly explained by the much longer hair exposure time for females than males due to the difference in sampling distance from the scalp.

  This study is not included in the registration dossier.

- Cequier *et al*. (2013) detected Dechlorane Plus in human serum samples (n=10) collected in Norway as part of a screening study. In a further paper, Cequier *et al*. (2015) measured serum concentrations of several flame retardants (including Dechlorane Plus) in 46 Norwegian women. The cohort was established in 2012 but the date of sample collection is not reported. Concentrations were measured by GC–MS (electron capture negative ionization mode). The syn- isomer was detected in 78 % of samples, at a median, mean and maximum concentration of 0.45, 0.77 and 6.7 µg/kg lw, respectively. The anti- isomer was detected in 89 % of samples, at a median, mean and maximum concentration of 0.85, 1.8 and 25 µg/kg lw, respectively. The total concentrations of both isomers appear to be broadly the same as the total PBDE concentrations in the same samples. The study also investigated associations between serum concentrations and measured indoor air and dust concentrations as well as diet and household factors, but this did not specifically include Dechlorane Plus.

  This study is not included in the registration dossier.

- Sahlström *et al*. (2014) measured levels of various flame retardants in serum samples from 24 first-time mothers who had delivered in Uppsala, Sweden in 2009−2010 and their matched children (aged 11−15 months). Analysis was by GC-MS. Anti- and syn-Dechlorane Plus were detected in serum from one toddler (at a concentration of 85 and 63 µg/kg lw, respectively) and one mother (49 and 39 µg/kg lw, respectively) from different households. The difference in results compared to Norwegian people (as reported by Cequier *et al*., 2013) may be due to higher detection limits (the detection limit was 85 and 41 pg/serum sample for the anti- and syn-isomer, respectively).

  This study is not included in the registration dossier.

- Brasseur *et al*. (2014) analyzed 48 banked human serum samples (24 male and 24 female, mean age 57±13 years, age range 28-86 years) collected between 2003 and 2005 from people living in the area of a municipal solid

waste incinerator in Besançon, France. Dechlorane Plus was detected in almost all the samples (detection frequency 94 % for the anti- isomer, LoD: 0.16 µg/kg lw) at a mean, median and maximum concentration (total isomers) of 1.40, 1.20 and 7.04 µg/kg lw, respectively. For comparison, BDE-47 and BDE-153 were the major PBDE congeners in the samples with mean levels of 2.06 and 1.39 µg/kg lw, respectively. There was no statistically significant difference in levels between males and females, nor correlation with age. The mean f_{anti} calculated for 33 samples was 0.75 (range: 0.65 to 0.86).

This study is included in the registration dossier as supporting monitoring information and is considered fully reliable by the Registrant.

- Siddique *et al*. (2012) collected 87 breast milk samples from two Canadian cities, i.e. Kingston (n=39, during 2003–2004) and Sherbrooke (n=48, during 2008–2009). Kingston is located on the north-east shore of Lake Ontario and Sherbrooke is about 400 km north-east of Kingston. At least one Dechlorane Plus isomer was detected in 91 % of the samples, with mean and median concentrations (total isomers) of 0.98 and 0.60 (range: not detected-8.0) µg/kg lw, respectively. These were up to ten times lower than those of concurrently measured major PBDEs, although a Principal Components Analysis indicated that the PBDEs came from a source that was distinct from that of Dechlorane Plus. Over 75 % of the samples had total Dechlorane Plus levels below 1 µg/kg lw, and only 5 % had levels exceeding 3 µg/kg lw. There was little difference in the measured levels between the two cities. The f_{anti} was similar to that in commercial products (mean: 0.67).

  This study is not included in the registration dossier.

- Zhou *et al*. (2014) measured Dechlorane Plus in milk (n=105) and maternal serum (n=102) samples collected from mothers in the Sherbrooke area of Québec, Canada, between December 2007 and December 2009. Detection frequency in serum (77 % and 87 % for the syn- isomer and anti- isomer, respectively) was higher than that in milk samples (40% and 50%). The median total isomer concentrations were 2.37 µg/kg lw (range: <0.16 – ~70[75] µg/kg lw) in serum and 0.02 µg/kg lw (range: <0.03 – ~15 µg/kg lw) in milk. There was no evidence of stereoselective bioaccumulation.

  This study is not included in the registration dossier.

[75] The study reports data for the anti- and syn- isomers separately and only gives a median value for the total concentration. The maxima cited here result from adding the highest reported concentrations for the two isomers, although this might be misleading if they did not come from the same samples.

4 HUMAN HEALTH HAZARD ASSESSMENT RELEVANT FOR THE PBT ASSESSMENT

4.1 Toxicokinetics

4.2 Repeated dose toxicity

4.2.1 Repeated dose toxicity: oral

The data included in the registration dossier by the Registrant for repeated dose toxicity via the oral route are summarised as follows:

- A 90-day study in Brown Rat (*Rattus norvegicus*) (Oscarson *et al.*, 1975) is available, and considered reliable by the Registrant. It was conducted according to a protocol equivalent to OECD TG 408 in which the test material was administered to rats in feed. The NOAEL was ≥100 000 ppm based on the lack of substance related effects. The NOAEL was based on the highest dose tested. No further details were available on substance purity.

- A reliable 28-day oral (gavage) study in rats conducted according to the OECD TG 422 reports no test substance-related effects at any dose level (Schroeder, 2008). A NOEL of ≥5 000 mg/kg bw/day is reported, based on the highest dose tested. The registration dossier states that an analytical certificate on substance purity was included in the original report. No further detail is included in the IUCLID robust study summary, although based on the available information (expressed as a percentage, but without detailed information on composition) the purity appears to be the same as the registered substance.

- Li *et al*. (2013b) performed a non-standard 90-day oral study with rats. Dechlorane Plus was administered mixed in corn oil by oral gavage for 90 days, and its distribution to different tissues, gene expression, clinical chemistry parameters and enzyme activity in liver were investigated. The results relevant to repeated dose toxicity include a significant ($p < 0.05$) decrease in alanine amino transferase (ALT), alkaline phosphatase (ALP), total bile acids (TBA) and glucose levels. No effect on measured serum thyroid hormone levels was detected. The study does not give full detail on observations, but states that no systemic toxicity was evident. The study includes endpoints (gene expression, enzyme activity, distribution) not included in a standard OECD TG 408 study, but does not report full histopathology or observations, and the group size per dose is less than that specified in OECD TG 408. The Registrant considers this study to be reliable with restrictions (shortcomings compared to OECD 408 guideline include limited number of animals, maximum dose of 100 mg/kg bw/d and it remains unclear whether no effects were seen or whether such further observations and endpoints on histopathology were not made).

Another study is available in the open literature that has not been considered by the Registrant:

- Wu *et al*. (2012) exposed mice (*Mus musculus*) to Dechlorane Plus to assess its effects on biomolecular parameters in the liver. Twenty-four six-week-old male mice were randomly allocated to four equal groups, which were then exposed by gavage daily to either corn oil (the control) or Dechlorane Plus in corn oil at 500, 2 000 or 5 000 mg/kg bw for a period of 10 days. Livers were removed after sacrifice and analysed for signs of oxidative stress, DNA adduct

formation and oxidative DNA damage, DNA strand breaks, gene expression and hepatic metabolites.

There were no significant treatment-related changes in body weight or relative weight of kidneys and testicles, but an increase in relative liver weight was observed, which was statistically significant for the 2 000 mg/kg treatment group ($p < 0.05$). The results indicate that after 10 days' oral exposure, oxidative stress and damage was induced in male mouse livers at 500 mg/kg bw and above, with altered hepatic carbohydrate, lipid, nucleotide and energy metabolism as well as signal transduction processes. However, DNA strand breaks did not occur. The study authors concluded that Dechlorane Plus may cause liver impairment. There is no framework to assess the significance of these findings in relation to the Annex XIII criteria, but the results are interpreted to mean that a toxic effect cannot be excluded over longer timescales.

4.2.2 Repeated dose toxicity: inhalation

A 28-day study, conducted according to a protocol equivalent to OECD TG 412, is included in the registration dossier and considered reliable by the Registrant (Hooker Chemical Corporation, 1975). No details are available on substance purity or composition. Analytical verification of test concentrations was carried out. A NOAEC of 1.524 mg/L air (dust, highest concentration tested) is reported in male and female animals based on increased liver weights due to hepatocellular hypertrophy, increased lung weights and slight diuresis. The observed effects are described by study authors to be due to functional adaptation.

4.2.3 Repeated dose toxicity: dermal

A 28-d repeated dose toxicity study in rabbits was conducted according to a protocol equivalent to OECD TG 410 (Tryzna *et al*., 1975). The NOAEL is reported to be >2 000 mg/kg bw/day based on the observation of no evident systemic effects (including on organ weights) and minimal, barely perceptible local irritation in the animals, at the highest dose tested. No information is available on the purity or composition of the test material.

4.2.4 Summary and discussion of repeated dose toxicity

Reliable data are available via the oral, inhalation and dermal routes for repeated dose toxicity. No specific target organ toxicity is reported in any of the available studies, and clinical signs are described as minimal. Some changes in biochemical parameters in liver tissue in both rats and mice have been reported following oral exposure, and although these might not be adverse, a toxic effect cannot be excluded over longer timescales. It is not known whether the substance is completely soluble in the corn oil vehicle. If the substance was present as microcrystals, then actual exposure to dissolved substance might have been more similar across the dose range.

The substance is not classified for repeated dose toxicity by the Registrant.

4.3 Mutagenicity

4.3.1 *In vitro* data

The Registrant reports two reliable studies, which are stated to have been conducted according to protocols equivalent to the current OECD test guidelines 471 and 476 for the genotoxicity endpoint.

Dechlorane Plus was tested in a reverse bacterial mutation assay with *S. typhimurium* strains TA 98, TA 100, TA 1535, TA 1537 and TA 1538, in the presence and absence of metabolic activation (Mortelmans *et al*., 1980). No evidence of mutagenicity is reported. No precipitation is reported at any of the dose concentrations up to 5 000 µg/plate with DMSO used as solvent. No information on the purity of the substance was available.

A study of mammalian mutagenicity is also available. Dechlorane Plus was tested in a mouse lymphoma assay using L5178Y cells, in the presence and absence of metabolic activation at dose concentrations up to 10 and 20 µg/mL respectively, with no higher concentrations included due to precipitation (Jotz *et al*., 1980). DMSO was used as a solvent. The test found Dechlorane Plus negative for mutagenicity in the presence and the absence of metabolic activation, with appropriate negative and positive controls in place. No information on the purity of the substance was available.

In addition, a non-standard study on DNA damage and repair in bacteria is available which yielded ambiguous results and from which it was not possible to draw a conclusion (Mortelmans *et al*., 1980). The study used *S. typhimurium* strains SL4700/SL4525 and TA 1538 / TA 1978 in order to detect a difference between growth inhibition zones in repair deficient and repair proficient strains. No growth inhibition was detected in either, for which reason the results are ambiguous.

4.3.2 *In vivo* data

xii) A 'reliability 3' (unreliable) *in vivo* study is included in the data set, which is not suitable for assessment of genotoxicity due to ambiguous results (Mortelmans *et al*., 1980). The method used the Ames test for gene mutation, with the urine of treated rats. No increase in mutant frequency was observed in response to urine from rats treated with the test material in any strain (TA 98, TA 100, TA 1535, TA 1537, TA 1538) at any concentration with or without metabolic activation. Negative controls were clearly negative, but positive controls were only partly and moderately positive. It was not demonstrated whether the urine of treated rats contained the test substance or its metabolites.

4.3.3 Summary and discussion of mutagenicity

Reliable bacterial and mammalian mutagenicity studies, both conducted according to protocols equivalent to current guidelines, are included in the dataset for genotoxicity. The substance is not mutagenic in either the presence or the absence of metabolic activation (Mortelmans *et al*., 1980; Jotz *et al*., 1980). The studies form the basis of the chemical safety assessment for genotoxicity. A disregarded *in vivo* study is also included in the data set, as well as a second non-standard bacterial study with ambiguous results which do not contribute to the assessment (Mortelmans *et al*., 1980).

The substance is classified by the Registrant as not mutagenic based on the available data.

4.4 Carcinogenicity

4.4.1 Summary and discussion of carcinogenicity

In accordance with Annex IX requirements the Registrant has not included data for carcinogenicity in the registration dossier.

4.5 Toxicity to reproduction

4.5.1 Effects on fertility and development

A combined repeated dose and reproductive/developmental toxicity screening study was included in the registration dossier by the Registrant (Schroeder, 2008). This study appears to have been published by Brock *et al*. (2010). The study was conducted according to OECD TG 422. The test material was administered in corn oil to rats by oral gavage. No further detail is included in the IUCLID robust study summary on substance purity, although based on the available information (expressed as a percentage, but without detailed information on composition) the purity appears to be the same as the registered substance.

The reproductive indices assessed included viable and non-viable foetuses, late and early resorptions, number of corpora lutea, body weights and sex of foetuses, gross and visceral malformations and variations. The offspring were also observed for litter size, the number of stillborn and live pups, sex, bodyweights, daily behaviour and survival. Post-natal observation was carried out up until day 4 after birth. The parental animals were observed and examined in detail for clinical signs, body weights and food consumption, and copulation and oestrus cycle assessment were made for females. Sperm parameters were not examined in males. The parental and F_1 NOEL were both determined to be ≥ 5 000 mg/kg bw/day, with no evident treatment-related effects in any examined parameters for repeated dose or reproductive/developmental toxicity.

The study for developmental toxicity / teratogenicity is waived by the Registrant as being scientifically unjustified, because the developmental screening study meets the requirements of OECD TG 414, and no toxic effects on embryo-foetal development or maternal function were observed up to 5 000 mg/kg bw/day.

4.5.2 Summary and discussion of toxicity to reproduction

A combined reproductive and developmental toxicity screening study is included in the dataset. The developmental toxicity/teratogenicity study is waived by the Registrant on the grounds that the available OECD TG 422 screening study is sufficient to meet the requirements of the full OECD TG 414 study, required for Annex IX.

4.6 Summary of mammalian toxicity

Several of the mammalian tests were conducted by Industrial Bio-Test Laboratories many years ago. This test facility is known to have fabricated study results historically. U.S. EPA Certificates of Compliance for the submitted studies are required to fully validate the results. However, this does not affect the studies that are relevant for PBT assessment.

The substance does not appear to cause relevant adverse effects via oral or dietary exposure at concentrations above 1 000 mg/kg bw/d, although there are some data

gaps (e.g. there are no long-term studies exceeding 90 days, which might be important given the apparently slow uptake of the substance). The dosing vehicles might also limit exposure (e.g. due to the presence of undissolved micro-crystals), such that the high "doses" might not truly reflect the degree of exposure of the organisms.

Nevertheless, based on the available data, Dechlorane Plus does not meet the classification criteria for mutagenicity, toxicity to reproduction or specific target organ toxicity. The data are considered to be conclusive but not sufficient for classification for these endpoints. Carcinogenicity data are lacking (and are not required at the registration tonnage). There is some evidence for potential liver impairment in mice (Wu *et al*., 2012), but the significance of these findings is unclear.

5 ENVIRONMENTAL HAZARD ASSESSMENT

5.1 Aquatic compartment (including sediment)

5.1.1 Pelagic toxicity

5.1.1.1 Fish

5.1.1.1.1 Short-term toxicity to fish

A short-term fish toxicity study is available in the registration dossier (Russell, 1973). Bluegill fingerlings (Latin name not given, presumably *Lepomis macrochirus*) were exposed to a range of nominal concentrations between 6.25 to 100 ppm [mg/L] of commercial Dechlorane Plus, plus the control, for 96 hours in a flow-through regime. The study has been assigned reliability 2 by the Registrant (who indicates that the substance purity was comparable with the registered substance) and no guideline was followed. The test substance was administered by injecting a suspension up to 2 % acetone in the test medium. No analytical monitoring was carried out and visible precipitation was present at the bottom of the tanks. The pH of the test system was reported to be 9.4-9.5. There were no effects on behaviour or mortalities at any of the concentrations tested. The EC0 is said to be >100 mg/L. Although the study has been assigned reliability 2 by the Registrant they also state that the study is inconclusive due to the low water solubility of the test substance and the observed precipitation.

No significant adverse effects on hatchability, survival or malformations were reported at concentrations up to around 0.3 mg/L (300 µg/L) in three non-standard Zebrafish (*Danio rerio*) embryo toxicity studies that had similarities to OECD TG 236 (Noyes *et al*., 2015; Kang *et al*., 2016; Chen *et al*., 2017) (see Section 5.1.1.1.2 for full details). It is possible that the egg membrane presents a barrier to the passage of Dechlorane Plus given its low water solubility and high log K_{ow}, although one of the studies did report uptake as well as biological responses in the fish 6 days after fertilization. A fourth study (Hang *et al*., 2013 [ABST]) reported malformations, but few details are available so its reliability cannot be assessed.

A 96-h LC_{50} >1.3 mg/L and a 14-d LC_{50} >1.2 mg/L have been reported for Medaka *Oryzias latipes* by the Japanese Ministry of Environment (MoE, 2013[76]), following

[76] Also http://www.safe.nite.go.jp/english/sougou/view/ComprehensiveInfoDisplay_en.faces.

OECD TG 203 and 204 respectively. Only a summary of the results are available. The studies were conducted in 1995 and it is likely that dispersants were used. Ideally, the original study reports should be reviewed to confirm the validity of the study results, but as the studies were conducted at concentrations far exceeding the solubility limit of the test substance (< 2 ng/L) they suggest no effects over short-term exposures. This study is not mentioned in the registration dossier.

Non-test data

Predictions of short-term toxicity to fish using ECOSAR v.1.1 (Mayo-Bean, 2012) are valid for substances with a log K_{OW} up to 5 (neutral organics model) or 6 (vinyl/allyl halides model). As Dechlorane Plus has a log K_{OW} of ≥9, it falls outside of the prediction domain of the models, and no toxicity is expected at saturation.

Appendix 8 summarises aquatic toxicity data for four structural analogues. Neither of the closest analogues (Dechlorane 603 and Chlordene Plus) are predicted to be acutely toxic to fish up the limit of their water solubilities. Two further analogues (chlordane and heptachlor) are acutely toxic to fish, but are much less hydrophobic than Dechlorane Plus. Their log K_{OW} values exceed the prediction domain for the baseline fish acute model by about an order of magnitude. As neurotoxic pesticides, their mode of action may be very different too. They therefore cannot be used for direct read across purposes.

As neurotoxic pesticides, their mode of action may be very different too. They therefore cannot be used for direct read across purposes.

Discussion

The study in the registration dossier pre-dates OECD test guidelines and the methodology is not considered to follow current guidelines because the test concentrations are above the limit of solubility by at least three orders of magnitude and no analytical monitoring was carried out. The observed precipitation is likely to be due to the exposure concentrations of the study exceeding the limit of solubility at all test concentrations. The pH of the test system (9.4 to 9.5) was above the recommended range (pH 6.0-8.5 according to OECD TG 201).

Overall, the study of short-term toxicity to fish reported in the registration dossier is not reliable, but the findings (i.e. no acute toxicity up to the water solubility limit) are supported by a Japanese regulatory study, lack of significant effects in three non-standard Zebrafish embryo studies and from QSAR/analogue read across considerations. It is not necessary to conduct a new short-term toxicity study with fish based on the very low water solubility of Dechlorane Plus and its limited bioavailability. Low water solubility is a REACH Annex VIII Column 2 adaptation for this endpoint.

5.1.1.1.2 Long-term toxicity to fish

Two studies are included for this endpoint in the registration dossier by the Registrant.

Gara and Rawisina (1975) exposed Bluegill Sunfish (Latin name not provided, presumably *Lepomis macrochirus*) to nominal concentration of 0.1 ppm commercial Dechlorane Plus (used as a 0.001 % solution in acetone) for 30 days in a flow-through test system (substance purity was not reported). The study is not said to follow a guideline. No effects on behaviour or mortalities were observed. Analytical monitoring using Gas Chromatography (GC) was carried out on days 0, 6, 12, 18, 24 and 30. No details on the methods of the analysis are available and details on test methods are lacking (e.g. dosing system). The results are reported as a 30-day NOEC of >0.102 mg/L, based on initial measured concentrations. A BCF value

of 5.58 has been determined in the study based on measured concentrations of Dechlorane Plus in fish tissue. The Registrant has assigned the study reliability 2 and identified this as the key study for long-term fish toxicity, although it is described as a bioaccumulation study.

The second study (Boudreau and Rausina, 1973) is a 30-day static bioaccumulation study with Bluegill Sunfish (Latin name not provided, presumably *Lepomis macrochirus*) exposed to 1 % suspension of commercial Dechlorane Plus dissolved in acetone. The study has been determined to be non-reliable (reliability 3) by the Registrant because the test substance purity was not reported, the measured concentrations were significantly lower than nominal concentrations and there was likely to be precipitation of the substance and subsequent ingestion by the fish. No mortalities and no effects on behaviour are reported in the study.

In addition, a dietary bioaccumulation laboratory test by Tomy *et al*. (2008) found that exposure of juvenile Rainbow Trout (*Oncorhynchus mykiss*) to food dosed with either 0.79 µg/g lipid weight (lw) of the syn- isomer or 1.17 µg/g lw of the anti- isomer for 49 days caused no effect on growth rate, liver somatic index or mortality (see Section 3.4.1.2.2).

xiii) In a further study reported in the academic literature, Liang *et al*. (2014) exposed juvenile Chinese Sturgeon (*Acipenser sinensis*) (around 1 kg in weight) via a single intraperitoneal injection to Dechlorane Plus (purity > 95 %) at doses of 1, 10 and 100 mg/kg ww (with a control) for 14 days. The test substance was dissolved in corn oil, and the fish were fed with tubificid worms twice a day. After 14 days, liver proteomes from three fish per treatment group were analyzed using two-dimensional electrophoresis coupled matrix-assisted laser desorption/ionization tandem time-of-flight mass spectrometry. Quantitative spot comparisons were performed with image analysis software and approximately 740 spots were detected on each gel. Among these proteins, 39 protein spots were found to be altered in abundance (>2-fold) in one or more treatment groups compared to that of the controls. According to the ratio value, there were 12, 24, and 15 significantly altered spots for the 1, 10 and 100 mg/kg ww groups, respectively. Of the 39 significantly altered proteins, 27 were successfully identified. Proteins related to the stress response that included heat shock cognate protein 70 and T-complex protein 1 were significantly increased and decreased in abundance, respectively. Moreover, Ras-related protein Rab-6B and GDP dissociation inhibitor 2, proteins that are involved in small G-protein signal cascades, were decreased in abundance two- to five-fold. Annexin A4, which is associated with calcium ion signalling pathways, was also markedly decreased by two-fold in the liver. Pathway analysis of differentially regulated proteins revealed that Dechlorane Plus exposure may induce oxidative stress, cell proliferation and apoptosis, which may be mediated through the stress response, small G-protein signalling cascades, calcium ion binding and carbohydrate metabolism.

xiv) This study is included in the registration dossier and in the view of the DS considered to be reliable with restrictions.

A 21-d NOEC >1.2 mg/L for Medaka *Or. latipes* has also been reported by the Japanese Ministry of Environment (MoE, 2013[77]). Only a summary of the results is available. The study was conducted in 1995 and it is likely that dispersants were used. Ideally, the original study report should be reviewed to confirm the validity of the results and the test method. The study was conducted at concentrations far

[77] Also http://www.safe.nite.go.jp/english/sougou/view/ComprehensiveInfoDisplay_en.faces.

exceeding the solubility limit of the test substance (<2 ng/L) and therefore should be treated with caution. This study is not mentioned in the registration dossier.

*Recent studies with Zebrafish (*Danio rerio*)*

a) Hang *et al*. (2013) [ABST] reported the effects of Dechlorane Plus on Zebrafish embryos and adults. This academic study is not mentioned in the registration dossier and has not subsequently been published. The tests were carried out at 28 °C at a pH of 7.5. Groups of 20 embryos (>8 hours post fertilisation (hpf)) were exposed to Dechlorane Plus for 7 days using a semi-static method. The exposure concentrations were 0.031, 0.074, 0.184 and 0.370 mg/L and acetone was used as a cosolvent (1 % v/v). The embryos were monitored daily for signs of malfunctions and mortality. Hang *et al*. (2013) [ABST] reported that the exposure resulted in the induction of observable spine side curve, cardiac adema and tail deformation in embryos and larvae and both dose-dependent and time-dependent effects on the total malformation rate were seen. Few other details of this part of the study are given so its overall reliability cannot be assessed. It should be noted that the concentrations used in this test are well in excess of the water solubility of Dechlorane Plus and so the significance of the effects is unclear.

   The experiments with adults were carried out using dietary exposure. Six-month old adults were given doses of 250, 2 500 and 7 500 mg/kg bw/day for up to 28 days. The food used in the test was prepared by mixing powdered Dechlorane Plus with brine shrimp and agar in water, heating in a microwave for 30 seconds and then allowing the mixture to cool and solidify. Two control groups were used, one fed with brine shrimp only and the other fed with a mixture of brine shrimp and agar. The accumulation of Dechlorane Plus in the fish was assessed after 7 days' exposure (additional exposure groups of 500 and 10,000 mg/kg bw/day appear to have been used). The concentrations measured in the fish at day 7 were 0.618, 0.587, 0.763, 1.823 and 1.879 mg/kg for the 250, 500, 2 500, 7 500 and 10 000 mg/kg bw day exposure groups, respectively. The fraction of the syn-isomer (f_{syn}) was 0.36-0.49.

   The toxicity of Dechlorane Plus to the adults was assessed using a superoxide dismutase (SOD) assay, an apoptosis assay, gene expression analysis and proteomic analysis (of livers and brains from three replicates):

   - SOD activity was significantly increased at 28 days compared to controls in a dose-dependent manner (the increase was statistically significant $p<0.01$) at all three concentrations tested (250, 2 500 and 7 500 mg/kg bw/day). It was hypothesised that this increase was a result of accumulation of superoxide radicals and hence an increase in SOD activity to scavenge these radicals.

   - Cell apoptosis was investigated in intestinal tissue. Cell apoptosis was evident after 7, 14 and 28 days' exposure to 7 500 mg/kg bw/day with no detectable apoptosis evident at 250 and 2 500 mg/kg bw/day.

   - For the gene expression assay, mRNA expression levels of SOD and p53 (apoptosis pathway-related) genes were significantly changed following Dechlorane Plus exposure compared to controls. Expression of SOD was down-regulated at low concentrations and short exposure periods, but up-regulated at high concentrations for longer periods. This was thought to be related to induction of superoxide radicals by Dechlorane Plus. In addition p53 expression decreased significantly after 28 days exposure to 7 500 mg/kg bw/day.

- The proteomic profiles of zebrafish liver and brain were also found to have significant alternations after Dechlorane Plus exposure.

b) As part of a study of 44 flame retardants, Noyes *et al*. (2015) exposed dechorionated Zebrafish embryos from 6 to 120 hpf to five aqueous concentrations of Dechlorane Plus spanning four orders of magnitude (nominally 0.064 nM to 64 µM) [equivalent to 0.42 – 42 000 µg/L], plus a control. This academic study is not mentioned in the registration dossier. The test solution preparation method is not described in detail but DMSO was used as a co-solvent at 0.64 %. It appears that the study used static exposure. The embryos were maintained at at 28 °C but water quality parameters were not reported in the paper. Thirty-two replicates (1 embryo/well) were used for each concentration. At 24 hpf, embryos were evaluated for survival, delays in developmental progression, notochord deformities, and altered spontaneous movements. Embryos that did not move (no body flexions, tail contractions) after 60 s were scored as having altered spontaneous movements. At 120 hpf, larvae were evaluated for survival and seventeen developmental malformations, including yolk sac edema and pericardial edema; body axis, trunk length, caudal fin, pectoral fin, pigmentation, and somite deformities; eye, snout, jaw, and otolith malformations; gross brain development; notochord and circulatory deformities; swim bladder presence and inflation; and touch responses (to test for normal rapid swimming and touch-escape responses).

Neurobehavioral changes were measured using two photomotor response assays with embryos at 24 hpf (spontaneous body flexions/tail contractions) and larvae at 120 hpf (total movement). Overall patterns of activity within each light interval were compared with those in vehicle controls. Embryos that were dead or malformed, including those with altered spontaneous movements, were not included. Sample sizes were 27 – 31 per concentration for the 24 hpf assay and 26 – 30 for the 120 hpf assay (there was no obvious dose-response relationship for mortality, with the highest test concentration achieving the highest survival rate).

No significant developmental malformations were reported and there were negligible effects on survival in this study. No effects on photomotor response were observed at 24 hpf. At 120 hpf, a significant hyperactive response was measured in the dark startle phase compared with controls at the highest Dechlorane Plus test concentration (which was several orders of magnitude higher than the solubility limit in pure water). The level of response was much lower than phosphate ester and chlorinated phosphate ester flame retardants in the same test system.

c) Kang *et al*. (2016) exposed adult male Zebrafish to Dechlorane Plus dissolved in corn oil at concentrations of 0, 0.02, 0.07 or 0.20 µg/µL [g/L] over 6 days. This academic study is not included in the registration dossier. The actual number of fish and replication per treatment is not reported so its reliability is unknown. Delivery was via gavage feeding with a syringe and catheter on two occasions (on day 0 and 2), using cold water (10-15 °C) to reduce fish activity beforehand and placing the fish in a groove within a sponge to keep it still. Nominal fish doses corresponded to 0, 0.3, 1 or 3 µg/g [mg/kg] ww. If corn oil leaked from the mouth or gill, the gavage feeding was repeated. While there is a possibility of overdosing for these fish, effects on the experimental results were considered minimal as the proportion was low (9 %). After dosing, the fish were placed in 10 L of dechlorinated tap water and fed with *Artemia* nauplii twice a day and kept at 26 °C under a 15:9 h light:dark photoperiod. Water was renewed every other day. On day 6, brain and testis were collected and

evaluated for thyroid- and sex hormone-related gene expression (one fish per replicate, five replicates per treatment), and thyroxine (T4) levels were measured in blood (3-4 fish per replicate, three replicates per treatment). Liver antioxidant enzyme activities (SOD (indirectly) and catalase) were also measured to assess oxidative stress.

Approximately 5 % of the fish died, which was considered to be a consequence of unsuccessful dosing. The outcome of water quality parameter monitoring (dissolved oxygen, pH, temperature and conductivity) was not reported. Whole body (minus major abdominal organs) residue levels at the end of the study were 4.97 ± 0.06 (clean control), 30.0 ± 0.40, 44.4 ± 0.46 and 420 ± 13.3 ng/g [µg/kg] ww (based on pools of several fish), demonstrating that the fish had taken up the substance even though they had only been given two gavage doses over a 6-d period.

Hepatic catalase activity significantly increased at the highest dose, but there was no effect on SOD. Average plasma T4 concentrations appeared to increase in a dose-related manner, and were 31 % higher than controls at the top dose, but the change was not statistically significant. Up-regulation of corticotropin-releasing hormone (*crh*) and thyroid stimulating hormone *β* (*tshβ*) genes (which regulate thyroid hormone synthesis) occurred in a dose-dependent manner in the brain, with a statistically significant change at the highest dose. However, transcriptions of other thyroid hormone-related genes (deiodinase 1, deiodinase 2, thyroglobulin (*tg*), thyroid hormone receptor *α* and *β*) were not altered in brain tissue.

Whilst some trends were apparent in up- or down-expression of genes related to sex hormone regulation in brain tissue, these were either not statistically significant (i.e. gonadotropin-releasing hormone 2 (*gnrh2*) and 3 (*gnrh3*), follicle stimulating hormone β (*fshβ*), luteinizing hormone β (*lhβ*), oestrogen receptor 2β (*er2β*) and androgen receptor (*ar*)), or non-monotonic (oestrogen receptor α (*erα*) was significantly up-regulated at the lowest exposure dose but not the other two). Cytochrome P450 19b (*cyp19b*) was significantly up-regulated at all exposure doses.

Oestrogen/androgen receptor and steroidogenic genes (*erα*, *er2β*, *ar*, cytochrome P450 17 (*cyp17*) and 11a (*cyp11a*)) were evaluated in testis, but significant alteration in gene transcription was not detected.

d) Kang *et al*. (2016) also exposed embryo/larval Zebrafish to Dechlorane Plus in water at nominal concentrations of 0.4, 0.8 and 1.6 mg/L along with a clean water control and solvent control (acetone (0.4 % v/v)). Six replicates were used per treatment, with 20 eggs within 4 hpf for each replicate. Test media were renewed and water quality parameters (dissolved oxygen, pH, temperature and conductivity) recorded every other day (but not reported). Chemical analysis was performed on fresh and expired test media, indicating actual concentrations of 140 - 28.8, 248 - 70.5 and 267 - 71.7 µg/L over the 48-h renewal period (no substance was detected in the controls). After 6 days, time to hatch, malformation rate (yolk sac edema, bent spine and tail malformation) and survival were recorded. Fourteen pooled larvae were also analyzed for expression of four genes related to thyroid hormone regulation (*crh*, *tshβ* and *tg* as for the oral gavage test above plus thyroid stimulating hormone receptor (*tshr*)) for each treatment. Body residues could not be measured due to low sample mass.

No significant effects on hatching time, survival or malformations were observed in any Dechlorane Plus treatment group compared to the solvent control (time to hatch was significantly delayed in the solvent control compared to the water control group). Transcriptional changes related to thyroid hormone regulation were not observed.

e) Chen *et al*. (2017) investigated developmental neurobehavioral toxicity in embryo-larval stages of Zebrafish. This academic study is not included in the registration dossier. The tests were carried out at 28 °C and a pH of 7.0-7.5. Normal fertilized embryos (from 6 hpf) were exposed to nominal aqueous Dechlorane Plus concentrations of 15, 30 and 60 µg/L for up to 120 hpf (5 days) using a semi-static regime (solutions renewed every other day). Three replicates were used per treatment (the total number of embryos per treatment is not clearly stated, but appears to have been at least 120 based on the supplemental data for mortality and hatching rate). Dimethyl sulfoxide (DMSO) was used as a cosolvent (0.1 % v/v), and a solvent control was included. Larval teratology, motor activity (assessed between 25 and 96 hpf using 60-72 embryos per treatment), motoneuron axonal growth (at 48 hpf and 10 embryos per replicate, n=3 replicates per treatment) and muscle morphology (at 120 hpf, 30 embryos per treatment) were assessed. Cell death (apoptosis) (12 embryos per treatment), reactive oxygen species (ROS), malondialdehyde (MDA) formation (a lipid peroxidation product) and axonal growth-related gene expression were also measured at 96 hpf.

Chemical analysis included spiking of samples with a recovery standard (PCB-209), with $^{13}C_{12}$-PCB-208 used as an internal standard. Total Dechlorane Plus concentrations in water were 12.3 ± 0.7, 27.1 ± 1.9 and 58.7 ± 1.8 µg/L in the three treatment groups (representing 82, 90 and 97.8 % of nominal, respectively); the time of analysis is not provided. Fish concentrations (100 fish, n=3 replicates) at 120 hpf were 757.8 ± 29.2, 1 319.3 ± 32.1 and 2 148.6 ± 41.8 ng/g [µg/kg] dw in the three treatment groups.[78]

Malformations (e.g. yolk-sac edema, pericardial edema, axial spinal curvature and cyclops or absence of eyes) and effects on hatching rate and mortality were reported to be no different from controls at 120 hpf. However, Dechlorane Plus exposure did induce a range of responses:

- Control embryos initiated alternating side-to-side contractions at 22 hpf, gradually reaching a peak of 7 bends/min at 23 hpf, then fluctuated around this frequency for nearly 2 h. Embryos exposed to Dechlorane Plus exhibited a similar pattern of spontaneous movement at the beginning but showed increased frequency of bending with time, peaking at 24 hpf. Statistically significant increases in spontaneous movement were observed at 24 hpf in all Dechlorane Plus treatments, with the highest peak frequency of 8.3 ± 0.4 bends/min occurring in the highest treatment.

- The average swimming distance of larvae (touch-induced movement) decreased in all Dechlorane Plus treatment groups compared to the control in a dose-dependent manner, but this was only statistically significant for the highest concentration at 48 hpf larvae, and the top two concentrations at 72 hpf.

- Free-swimming activity was assessed at 120 hpf, following a 24-h period in untreated medium. Significant decreases in free swimming speed were observed in the top two treatment groups, suggesting the existence of motor defects. Locomotion activities of larvae were also evaluated using

[78] Although this was a concentration-dependent increase and fish were rinsed with test medium before analysis, it is possible that these reflect adsorbed substance as well as uptake. Together with the short exposure duration and life-stage, these measurements are not considered relevant for bioaccumulation assessment, although they do suggest that uptake can occur relatively rapidly.

a 25-min alternating dark-to-light photoperiod stimulation. In controls, the swimming speed usually decreased rapidly in the light (increasing in the dark). Treated groups followed the same pattern, but the average swimming speed of larvae exposed to Dechlorane Plus was significantly lower than that of controls during the first 5-min dark period. Exposure to the highest concentration significantly decreased the average swimming speed during every dark period and the first light period.

In line with these behavioural anomalies, Dechlorane Plus significantly inhibited axonal growth of primary motoneurons in a dose-related way (with significant up-regulation of two nerve-related mRNA transcript levels at the highest concentration at 96 hpf). Apoptotic cell death in the tail region was observed in all treatments at 96 hpf (but not in controls), and a significant increase in ROS content, MDA level and mRNA transcript levels of two apoptosis-related genes (*bax* and *caspase-3*) were observed at the two highest treatments. Histological examination at 120 hpf showed that larvae exposed to the two highest concentrations had looser and more disordered arrays of muscle fibres in the tail compared with control larvae.

The study authors concluded that Dechlorane Plus induced neurobehavioral deficits which may result from combined effects of altered neuronal connectivity and muscle development, with associated oxidative stress; they speculated that nerve growth might have been affected by thyroid hormone disruption and/or intracellular calcium ion signalling (as suggested by the studies of Kang et al. (2016) and Liang et al. (2014)).

Non-test data

Predictions of long-term toxicity to fish using ECOSAR v.1.1 (Mayo-Bean, 2012) are valid for substances with a log K_{OW} up to 8. As Dechlorane Plus has a log K_{OW} of ≥9, it falls outside of the prediction domain of the models, and no toxicity is expected at saturation.

Appendix 8 summarizes aquatic toxicity data for four structural analogues. There is a lack of relevant structural analogues in the training sets of the models, which limits their reliability. The closest analogues (Dechlorane 603 and Chlordene Plus) are predicted to have a long-term fish NOEC around the limit of their solubility in water. However, both of these substances have predicted log K_{OW} values that exceed the cut-offs for the models by an order of magnitude or more, so these predictions cannot be considered reliable (i.e. no effects at saturation are expected for this endpoint). Two further analogues (chlordane and heptachlor) are chronically toxic to fish with predicted NOECs around or below 0.01 mg/L, but are much less hydrophobic than Dechlorane Plus. As neurotoxic pesticides, their mode of action may be very different too. They therefore cannot be used for direct read across purposes.

It should be noted that these predictions do not allow any comparison to be made about likely toxicity arising from dietary exposure.

Discussion

The Registrant has reported two bioaccumulation studies rather than an appropriate OECD or equivalent test guideline study for long-term toxicity to fish. The results of the key study (Gara and Rawisina, 1975) are reported in terms of initial measured concentrations. The NOEC based on mean measured concentrations is >0.087 mg/L (compared to the initial measured concentration based NOEC value of >0.102 mg/L). However, this does not affect the overall conclusion of no toxicity at the highest dose tested. It is noted that the measured test concentration was still at least one thousand times higher than the expected solubility limit in water (< 2 ng/L). It is likely that precipitation of the test material,

adherence onto the food given and subsequent ingestion of the test material took place. In addition, the hydrophobic properties of the substance indicate that it is likely that it would have adhered onto the test vessel. The study does however indicate that no mortalities were reported for Bluegill Sunfish fingerlings exposed to the test substance, above its water solubility limit, for 30 days.

A 21-d NOEC >1.2 mg/L for *Or. latipes* (MoE, 2013) of unknown reliability also suggests a lack of toxicity over this timescale.

The Boudreau and Rausina (1973) study is considered to be unreliable by the Registrant. The study is reported as a 30-day static study, though it seems unlikely that this was the case because water quality parameters such as pH and oxygen would have deteriorated to levels comprising fish health at all treatment levels before the end of the study. No information on water quality is reported and no mortalities are recorded throughout the study. The mean measured concentration over the 30-day exposure period has been calculated to be 0.03 ppm [mg/L], which is over one thousand times higher than the water solubility limit of Dechlorane Plus (< 2 ng/L). It is likely that precipitation and subsequent ingestion of the test substance took place.

Bioaccumulation studies are not considered to fulfil the requirements for long-term toxicity to fish endpoints because although they may provide useful data on mortality (and behaviour, if recorded), they do not measure the more sensitive sub-lethal endpoints such as growth and reproductive parameters, and they test different life stages of an organism. In addition, the duration of a true long-term toxicity to fish studies tends to be longer than bioaccumulation studies, depending on the guideline and the species tested (e.g. fish early-life stage (FELS) studies with *Oncorhynchus mykiss* are conducted up to 60 days).

In principle, a long-term toxicity study conducted according to OECD TG 210 (FELS) would be appropriate for a substance with a log K_{OW} above 5. However, the properties of Dechlorane Plus (high log K_{OW} (≥9), very low water solubility (< 2 ng/L)) mean that the time needed to reach equilibrium in an organism (i.e. to bioconcentrate) is likely to be longer than the exposure time in a long-term aquatic toxicity study of standard duration (Hawker and Connell, 1986). This limited bioavailability means that standard long-term aquatic toxicity studies involving exposure via water are unlikely to indicate any significant effects. In addition, aqueous studies would be very difficult to perform because the substance will have a high tendency to adsorb to organic matter and glassware, making it difficult to maintain test concentrations (ECHA, 2008). On the other hand, Zebrafish embryo studies suggest that higher concentrations may be possible in test media, at least in small volumes such as well plates and with the use of co-solvents.

The distribution modelling of the substance undertaken for the purposes of this assessment supports this conclusion as it indicates that Dechlorane Plus would mostly end up in the sediment or soil compartments (see Section 3.2.3). In addition, its properties indicate that it would have a high affinity for organic carbon (see Section 3.2) and therefore would have a low dissolved concentration in the environment.

xv) Nevertheless, the studies of Zhang *et al*. (2011a) and Zeng *et al*. (2014a) (see Appendix 3 and Section 3.4.1.2.1, respectively) show that the substance can cross the blood-brain barrier and be passed from females to eggs in fish. Sensitive life stages and tissues are therefore exposed to the substance. The study by Liang *et al*. (2014) is difficult to interpret, especially as it involved intraperitoneal injection, which may lead to significantly higher concentrations in liver than is typical following dietary exposure (the liver concentrations were not reported). It suggests that a variety of effects can occur in fish liver at doses of 1 mg/kg ww and higher, but there is no information to link these to an overall adverse effect. This

study suggests that the substance might induce potentially toxic effects in the liver, but is inconclusive. However, it is noteworthy that similar findings of oxidative stress and other signs of molecular and/or tissue damage were also observed in studies with mice via oral exposure (see Wu *et al*. (2012) in Section 4.2.1) and earthworms (see Section 5.2).

It is not clear how closely the available Zebrafish embryo tests followed OECD TG 236 or whether the relevant validity criteria were met and so their reliability is uncertain. For example, the Chen et al. (2017) study used a slightly higher temperature than the OECD recommendation, did not provide information on water quality (e.g. dissolved oxygen concentration or hardness) and there is no mention of a positive control. However, control mortality and hatching rate were satisfactory (measured at 120 hpf rather than the recommended 96 hpf) and a higher number of fish per treatment were used than required by the OECD TG (apparently n=120 fish per treatment).

xvi) The standard fish embryo toxicity test is considered to be a short-term acute test, with hatch/mortality/malformation end points only. Neither the Noyes *et al*. (2015), Kang *et al*. (2016) nor Chen *et al*. (2017) studies detected significant effects for these endpoints. Whilst the observed developmental neurobehavioral effects in the Noyes *et al*. (2015) and Chen *et al*. (2017) studies may possibly indicate an adverse response (cyclodiene insecticides such as chlordane – which are analogues of Dechlorane Plus – are generally considered to be neurotoxic to terrestrial organisms), it is difficult to determine how they relate to population-level apical effects. Ideally, the degree and timings of the changes would be compared to reliable results obtained in the same test system for known neurotoxicants, so that an adverse outcome pathway could be developed. Noyes *et al*. (2015) detected a significant neurobehavioural effect at a very high nominal test concentration (ca. 40 mg/L), but the level of response was lower than other substances that are suspected of having a neurotoxic mode of action. Similarly, Chen *et al*. (2017) noted that the significantly elevated frequency of spontaneous movement induced by Dechlorane Plus at 24 hpf was different to that observed for two other substances in apparently similar test systems[79]. Chen *et al*. (2017) also noted that external factors such as temperature can affect spontaneous movement of Zebrafish embryos.

xvii) In the absence of a standardised method for such end points and reliable benchmarking against other substances, these studies raise a potential concern for fish but do not demonstrate an effect equivalent to the level of concern associated with the Annex XIII T criterion, particularly as the main effects were observed at or above 30 µg/L (nominal) (i.e. the NOEC from would seem to be >0.01 mg/L). It should also be noted that the concentrations used in the aqueous studies were all well in excess of the reported water solubility of Dechlorane Plus and so the significance of the effects is unclear.

xviii) Kang *et al*. (2015) exposed adult male Zebrafish over a 6-d period by gavage, and detected a non-significant increase in average plasma T4 concentrations and significant up-regulation of corticotropin-releasing hormone (*crh*) and thyroid stimulating hormone *β* (*tshβ*) genes in the brain, together with significant effects on hepatic catalase (though not SOD) activity, at the highest dose. Transcriptions of five other thyroid hormone-related genes were not altered. Responses from genes related to sex hormone regulation in brain and testis tissue

[79] 2,2',4,4'-Tetrabromodiphenyl ether (BDE-47) significantly increased the frequency of spontaneous movement from 20 to 23 hpf at 20 µmol/L [about 10 mg/L], whereas bisphenol-A significant decreased the frequency of spontaneous movement from 25 to 29 hpf at 15 mmol/L [3.4 g/L]. These are much higher concentrations than the reported water solubility of these substances cited in existing EU regulatory reports (around 11 µg/L and 300 mg/L, respectively) so these data might not be reliable.

were also investigated, but these were almost all either not statistically significant or not monotonic (one gene only). Only one gene (*cyp19b*) was significantly up-regulated in the brain at all exposure doses. Whilst these results demonstrate that uptake occurred and that there was a biological response in fish, the method of administration (which may have induced significant stress), variable findings and lack of any link to a population-relevant apical effect mean that their relevance cannot be assessed.

xix) Similarly, the recent study by Hang *et al.* (2013) [ABST] shows that Dechlorane Plus has biological activity in adult Zebrafish exposed via the diet over 7 days, but the nature of the effects (cell damage or apoptosis and SOD activity), and the relatively high concentrations/doses used (up to 7 500 mg/kg bw/day), means that it is difficult to assess the significance of these effects in terms of population survival in the environment.

xx) Due to the lack of reliable information on Dechlorane Plus itself, Government of Canada (2016) used a critical body residue (CBR) approach to assess the potential for acute and chronic toxicity to fish. Based on a series of assumptions (including the level of dietary accumulation in fish), it was concluded that Dechlorane Plus can not achieve a CBR associated with lethal effects as a result of baseline narcosis. This does not consider other modes of action (like developmental neurotoxicity).

Overall, long-term fish toxicity tests using aqueous exposure might not be relevant for this substance due to its very low solubility in water, which will limit its bioavailability. However, effects on fish following exposure via food cannot be ruled out given the high potential for bioaccumulation and indications of changes in liver proteins in fish and mammals (and oxidative stress and other signs of molecular and/or tissue damage in earthworms and Zebrafish). A dietary toxicity study might therefore be appropriate, although no guideline exists, and there is no guidance available to allow the interpretation of the results in the context of Annex XIII. The Tomy *et al*. (2008) dietary bioaccumulation study failed to find signs of toxicity over a reasonably long exposure period, although the actual exposure concentration (in terms of mg/kg feed) is unknown.

5.1.1.2 Aquatic invertebrates

5.1.1.2.1 Short-term toxicity to aquatic invertebrates

A short-term toxicity study for aquatic invertebrates has been waived by the Registrant based on the low water solubility of the substance.

A 48-h EC_{50} value of >1.3 mg/L based on immobilisation of *Daphnia magna* has been reported by the Japanese Ministry of Environment (MoE, 2013[80]), following OECD TG 202. Only a summary of the study result is available. The study was conducted in 1995 and it is likely that dispersants were used. Ideally, the original study report should be reviewed to confirm the validity of the study results. The study was conducted at concentrations far exceeding the solubility limit of the test substance (< 2 ng/L) and therefore should be treated with caution. This study is not mentioned in the registration dossier.

Non-test data

Predictions of short-term toxicity to *Daphnia* using ECOSAR v.1.1 (Mayo-Bean, 2012) are valid for substances with a log K_{OW} up to 5 (neutral organics model) or

[80] Also http://www.safe.nite.go.jp/english/sougou/view/ComprehensiveInfoDisplay_en.faces.

6 (vinyl/allyl halides model). As Dechlorane Plus has a log K_{OW} of ≥9, it falls outside of the prediction domain of the models, and no toxicity is expected at saturation.

Appendix 8 summarises aquatic toxicity data for four structural analogues. Neither of the closest analogues (Dechlorane 603 and Chlordene Plus) are predicted to be acutely toxic to *Daphnia* up the limit of their water solubilities. Two further analogues (chlordane and heptachlor) are predicted to be acutely toxic to *Daphnia*, but are much less hydrophobic than Dechlorane Plus. Their log K_{OW} values exceed the prediction domain for the baseline Daphnid acute model by about an order of magnitude; chlordane is outside the domain for the vinyl/allyl halides class prediction for Daphnid acute toxicity too. As neurotoxic pesticides, their mode of action may be very different too. They therefore cannot be used for direct read across purposes.

Discussion

As for fish, it is not necessary to conduct a short-term toxicity study with invertebrates based on the very low water solubility of Dechlorane Plus and its limited bioavailability. Low water solubility is a REACH Annex VII Column 2 adaptation for this endpoint.

5.1.1.2.2 Long-term toxicity to aquatic invertebrates

A long-term toxicity test with aquatic invertebrates has been waived by the Registrant based on the low water solubility of the substance, the lack of effects in short- and long-term toxicity studies with fish, and on the grounds that the substance is not bioaccumulative.

21-d EC_{50} and NOEC values of >1.3 mg/L based on reproductive effects with *Daphnia magna* have been reported by the Japanese Ministry of Environment (MoE, 2013[81]), following OECD TG 211. Only a summary of the results are reported. The study was conducted in 1995 and it is likely that dispersants were used. Ideally, the original study report should be reviewed to confirm the validity of the study results. The studies were conducted at concentrations far exceeding the solubility limit of the test substance (< 2 ng/L) and therefore should be treated with caution. This study is not mentioned in the registration dossier.

Non-test data

Predictions of long-term toxicity to invertebrates using ECOSAR v.1.1 (Mayo-Bean, 2012) are valid for substances with a log K_{OW} up to 8. As Dechlorane Plus has a log K_{OW} of ≥9, it falls outside of the prediction domain of the models, and no toxicity is expected at saturation.

Appendix 8 summarizes aquatic toxicity data for four structural analogues. There is a lack of relevant structural analogues in the training sets of the models, which limits their reliability. The closest analogues (Dechlorane 603 and Chlordene Plus) fall outside of the prediction domain of the models, so no toxicity is expected at saturation. Two further analogues (chlordane and heptachlor) are chronically toxic to invertebrates with predicted NOECs around or below 0.01 mg/L, but are much less hydrophobic than Dechlorane Plus. As neurotoxic pesticides, their mode of action may be very different too. They therefore cannot be used for direct read across purposes.

[81] Also http://www.safe.nite.go.jp/english/sougou/view/ComprehensiveInfoDisplay_en.faces.

Discussion

It is not necessary to conduct a long-term toxicity study with invertebrates based on the substance having very low water solubility and limited bioavailability, i.e. toxicity is not expected to be expressed in aquatic studies involving water-only exposure over 21 days. The other points forming the basis of the waiver are not appropriate for this substance (long-term toxicity to fish is unresolved and the substance is bioaccumulative).

5.1.1.3 Algae and aquatic plants

A study of toxicity to algae and aquatic plants has been waived by the Registrant based on low water solubility of the substance and observed precipitation in the short-term toxicity study with fish.

A 72-h ErC_{50} and $NOEC_{growth}$ >0.35 mg/L have been reported for *Pseudokircheneriella subcapitata* by the Japanese Ministry of Environment (MoE, 2013[82]), following OECD TG 210. Only a summary of the results are reported. The studies were conducted in 1995 and it is likely that dispersants were used. Ideally, the original study report should be reviewed to confirm the validity of the study results. The studies were conducted at concentrations far exceeding the solubility limit of the test substance (< 2 ng/L) and therefore should be treated with caution. This study is not mentioned in the registration dossier.

The effects of Dechlorane Plus on *P. subcapitata* has been studied using a non-standard test method involving flow cytometry[83] (Gong *et al.*, 2013 [ABST]). The test was carried out by exposing *P. subcapitata* (initial cell density 10^5 cells/mL) to nominal concentrations of Dechlorane Plus of 13.51, 135.1 and 1 351 ng/L for up to 72 hours. Acetone (<0.1 v/v) was used as a cosolvent. The effects of exposure on cell membrane integrity, esterase activity, intracellular reactive oxygen species (ROS) generation and chlorophyll a fluorescence were determined after 2, 24, 48 and 72 hours' exposure. Some initial dose-related damage to the cell membrane was seen at two hours but this was minor and the cells recovered with increasing exposure time. Esterase activity was found to be significantly increased compared with controls at two hours at concentrations of 135.1 and 1 351 ng/L, and at 24 hours at 135.1 ng/L but no significant induction was seen at any concentration at 48 and 72 hours. ROS generation was significantly increased at 48 hours at 13.51 and 135.1 ng/L but decreased at 1 351 ng/L. This was explained in terms of accumulation of intracellular ROS and induction of the antioxidant defences in the cells, resulting in scavenging of the free radicals. At 72 hours the ROS generation in the exposed cells was similar to that in the control cells. Overall, Gong *et al.* (2013) [ABST] concluded that the results indicated that Dechlorane Plus showed a low toxicity and had marginal effects at concentrations up to 1 351 ng/L. The concentrations tested in this study are well in excess of the water solubility of the substance. This study is not mentioned in the registration dossier.

An indication of potential toxicity of Dechlorane plus to Sea Lettuce (*Ulva pertusa*) has been reported by Zhao *et al*. (2014) as part of a bioconcentration study (see Section 3.4.1.2.1). The study was carried out by exposing pieces of the organisms (initial mean weight 0.46 g) to a Dechlorane Plus concentration of 1 351 ng/L for 21 days at 10 °C followed by a 14-day depuration period. Acetone (0.01 % v/v) was used as a cosolvent. Growth inhibition in exposed organisms compared to the

[82] Also http://www.safe.nite.go.jp/english/sougou/view/ComprehensiveInfoDisplay_en.faces.

[83] Flow cytometry is a method for measurement of the light-scattering and fluorescent properties of cells.

control was reported to have occurred on day 7 and 14 of the uptake phase. The specific growth rates for these two time points are given as -2.36 % per day and -0.97 % per day and these were statistically significantly different from the control group (p<0.05). The specific growth rate returned to similar levels as the control during depuration. This suggests that Dechlorane Plus may have exerted a toxic effect during the uptake phase but that these effects were reversible once exposure ceased. However, as noted in Section 3.4.1.2.1 the equation used to estimate the specific growth rate can be questioned. The study was not a standard method for evaluating toxicity, and in addition only a single concentration of Dechlorane Plus was tested – the initial concentration used exceeded the water solubility of Dechlorane Plus and the concentration was found to decline to <300 ng/L by day 7 of the uptake. This means that it is difficult to determine whether the effects seen (if genuine) were due to dissolved or undissolved test substance. No dose-response relationship information is available. In conclusion, although these results suggest that Dechlorane Plus exposure may have resulted in adverse effects in Sea Lettuce, they are not considered sufficiently robust to be used in hazard assessment.

Non-test data

Predictions of toxicity to algae using ECOSAR v.1.1 (Mayo-Bean, 2012) are in principle valid for substances with a log K_{OW} up to 6.4 (acute effects) or 8 (long-term effects). However, the basis for the vinyl/allyl halide model is weak and the training set for the neutral organics QSAR model also lacks suitable analogues for algal toxicity end points (see Appendix 8 for more discussion). Any predictions of algal toxicity within the prediction domain for this type of substance must therefore be considered potentially unreliable. Nevertheless, as Dechlorane Plus has a log K_{OW} of ≥9, it falls outside of the prediction domain of the models, and no toxicity is expected at saturation.

Appendix 8 summarises aquatic toxicity data for four structural analogues. Neither of the closest analogues (Dechlorane 603 and Chlordene Plus) are predicted to be acutely or chronically toxic to algae up the limit of their water solubilities. Two further analogues (chlordane and heptachlor) are predicted to be toxic to algae with EC_{50}/NOEC values in the range of approximately 0.1 – 1 mg/L. They are much less hydrophobic than Dechlorane Plus and their mode of action may be very different too. They therefore cannot be used for direct read across purposes.

Discussion

It is not necessary to conduct an algal inhibition study as toxicity is not expected to be expressed in aquatic studies over short durations due to the very low water solubility of Dechlorane Plus and its limited bioavailability. Low water solubility is a REACH Annex VII Column 2 adaptation for this endpoint.

5.1.1.4 Conclusions on aquatic toxicity for pelagic organisms

Although fully valid regulatory test data are not available for any end point, Dechlorane Plus is unlikely to express acute or chronic toxicity to aquatic organisms in standard tests involving exposure via water, in view of its very low water solubility (< 2 ng/L). However, effects on fish following exposure via food cannot be ruled out given the high potential for bioaccumulation and evidence to suggest that fish (as well as mammals and earthworms) can show indications of toxicity at a molecular level. A dietary toxicity study might therefore be appropriate, although no guideline exists, and there is no guidance available to allow the interpretation of the results in the context of Annex XIII.

5.1.2 Sediment organisms

No data have been presented by the Registrant for sediment organisms.

Discussion

A sediment organism toxicity study is not an Annex IX requirement (corresponding to the tonnage level of the registered substance) and no data were included in the registration dossier. The substance is expected to have a high affinity for organic carbon (see Section 3.2) and sediment may be an important compartment for the environmental fate of the substance, as indicated by the distribution modelling in Section 3.2.3.

No additional information on sediment toxicity has been found following a literature search. Due to the lack of reliable information on Dechlorane Plus itself, Government of Canada (2016) adopted a read-across approach using sediment organism toxicity test data for the analogue chlordane to screen for sediment hazards. The conclusion was that Dechlorane Plus has the potential to cause effects to sediment organisms at low concentrations. However, this is a very worst case approach, especially as the analogue was a pesticide (i.e. it assumes that Dechlorane Plus has the same mode of action). The ECHA Read Across Assessment Framework outlines a number of steps that need to be considered (such as bioavailability), which were not part of the Canadian assessment. Whilst acceptable for screening purposes, it is not appropriate to assume this information presents a realistic level of toxicity for Dechlorane Plus.

A further long-term sediment toxicity study could be performed, for which OECD TG 225 using spiked sediment would be most appropriate (since oligochaetes dwell within the sediment so oral exposure will potentially be higher than in other test species that live on or at the sediment surface).

5.2 Terrestrial compartment

No standard data are presented in the registration dossier for the terrestrial compartment. The terrestrial organism toxicity studies are waived based on the substance being insoluble, a lack of effects in the available aquatic toxicity studies, the lack of bioaccumulation potential and the fact that 90% of the substance is not absorbed according to available toxicokinetics studies.

The potential for effects of Dechlorane Plus exposure to soil microbiota has been studied by Thanh *et al*. (2013) [ABST]. The study is poorly reported and it is difficult to follow what was done exactly, and in many cases the significance of the reported effects is not clear. The tests used bacterial cultures of *Eschericha coli*, the dibenzofuran-degrading bacterium *Agrobacterium* sp. and the phosphorus solubilising bacterium *Gluconacetobacterliquefaciens* sp. Resting cells of the bacteria (10^6-10^7 colony forming units per mL) were added to pH 7.4 buffer medium or soil extract medium containing 250 ppb (~250 µg/L) of each Dechlorane Plus isomer and the solutions were incubated for up to 24 hours. Effects on cell viability were seen, with higher effects being seen with the syn-isomer than the anti-isomer. In addition an increase in reactive organic species (ROS) was also seen and appeared to be related to the decrease in colony forming units. No significant effects were evident on the phosphorus solubilizing and dibenzofuran degrading capacities of the bacteria.

Overall the results of this test are difficult to assess in terms of the possible effects of Dechlorane Plus on terrestrial ecosystems. This study is not included in the registration dossier.

The registration dossier includes a short-term toxicity study on the earthworm *Eisenia fetida* by Zhang *et al*. (2014). Healthy adult worms (60 days old, 300 – 400 mg, with well-developed clitellum) were used for all exposure experiments. The worms were acclimated in clean soil for one week. Test soils were prepared using

nominal Dechlorane Plus (purity >99 % w/w) concentrations of 0.1, 1, 10 and 50 mg/kg dw, plus an untreated control. Each treatment had three replicates and each 1 L bottle contained twelve worms. No details were provided about the preparation of the artificial soil (which contained 10% dried cow manure (as food), 20% kaolin clay, and 70% industrial sand; pH 6.0 ± 0.5) or dosing technique. The soil water content was 35 % (w/w) (in accordance with OECD TG 207) and water was regularly sprayed into the headspace of the bottles to keep the air humidity at 80% (the bottles were covered with plastic film that had been punched with small holes). The bottles were supplied with continuous light at 20 ± 1 °C for 14 days. At the end of the exposure period, the worms were extracted from the soil and placed in enclosed petri dishes for 24 hours on moist filter paper at 20 ± 1 °C to allow them to purge their gut contents.

No significant treatment-related changes in mortality or body weight were found ($p > 0.05$). However, analysis of superoxide dismutase, glutathione and malonaldehyde indicated that the worms showed signs of oxidative stress, and other damage was also recorded (inhibition of acetylcholine esterase activity, significantly increased levels of DNA strand breaks and changes in transcriptomic profiles related to neurotoxicity), although there was not always a clear dose-response relationship for some of the findings. These are not standard endpoints, and given the short duration of this study, they cannot be used for this assessment. Nevertheless, they suggest that adverse effects could potentially occur over longer exposures.

This study is included in the registration dossier and considered fully reliable by the Registrant.

Yang *et al*. (2014) exposed earthworms *Eisenia fetida* to nominal Dechlorane Plus concentrations of 0.1, 0.5, 6.25 and 12.5 mg/kg dw (plus control) in artificial soil for 28 days. This academic study is not included in the registration dossier. Actual exposure concentrations were not measured (although this is not currently a requirement of any OECD soil test guideline). All effects are therefore based on the initial nominal test concentrations.

For each concentration, the desired amount of test substance was dissolved in 10 mL dimethylsulfoxide (DMSO) and mixed in to a small quantity of fine quartz sand. DMSO was allowed to evaporate overnight and then the dosed sand was mixed thoroughly with pre-moistened artificial soil in a household mixer. Worms (approximately 60 days old) were allowed to acclimatise for one week in untreated artificial soil (10 % cow manure, 20 % kaolin clay, 70 % industrial sand (particle size not reported) and a 'small amount' of calcium carbonate to adjust the pH to 6.0 ± 0.5 (pH not monitored during test)) before exposure began. Thirty worms were exposed in each treatment (ten individuals in three replicates). The test chamber size, surface area and soil depth were not reported (glass bottles were used). Soil moisture content was adjusted to 35 % by the addition of distilled water (the maximum water holding capacity of the soil was not reported and moisture content was not subsequently monitored). The temperature was 20 ± 1 °C and the worms were exposed to a continuous light source rather than a 16:8 h light:dark cycle; the impact of this on the worms in unknown. Light intensity was not reported. The worms were not fed during the experiment.

At the end of the exposure period, earthworms were removed from the soil, placed in enclosed petri dishes for 24 h on moist filter paper to purge their gut contents and then assessed for mortality and body weight change. Three washed earthworms per treatment were also pooled for biomarker assays performed in triplicate to assess oxidative stress (i.e. glutathione (GSH) and malondialdehyde (MDA) content, and superoxide dismutase (SOD), glutathione peroxidise (GSH-Px) and catalase (CAT) activities), neurotoxicity (measuring the neurotransmitter acetylcholine esterase, AChE), cellulase activity (considered to be relevant to the

earthworms' ability to decompose plant litter) and DNA damage (using an invertebrate 8-hydroxy-2'-deoxyguanosine (8-OHdG) enzyme-linked immunosorbent assay (ELISA) kit and a Comet assay). Enzyme activities were standardized by protein content.

No mortality (greater than the 10% validity criterion in OECD TG 222) was observed in the control group. There were no significant treatment-related changes in mortality ($p > 0.05$) in any of the treatment groups. No significant treatment-related changes in body weight were found at any test concentration in comparison with controls. 'Negative growth' of earthworms appeared simultaneously in control and treated groups, which was suggested to be due to the change from natural to sub-optimal artificial soil (a drop in body weight is not uncommon in worms exposed in artificial soil, especially if they are not fed throughout the exposure period). Overall the study authors reported that the direct toxicity to earthworms was 'very low'.

A number of changes in biomarkers were reported:

- SOD and GSH-Px activities significantly ($p < 0.05$) decreased in a dose-related manner compared to the control. CAT activity was also inhibited significantly at concentrations over 0.5 mg/kg dw. GSH levels were inhibited significantly compared to controls at lower concentrations (0.1 and 0.5 mg/kg dw) but also significantly increased at higher concentrations (6.25 and 12.5 mg/kg dw). The authors considered these changes to be potentially indicative of environmental stress. The changes in enzyme activity were not substantial given the scale of the increases in exposure concentration (there was little change between each of the two lowest and two highest concentrations). This might reflect limited bioavailability (i.e. actual exposure concentrations changing by less than suggested by the nominals), but this is conjecture. The actual meaning of these changes for earthworm populations is unclear.

- MDA and 8-OHdG were characterised as two types of oxidative damage for membrane lipids and nucleic acids respectively. There was a significant decrease ($p < 0.05$) in MDA content compared with the control. The level of decline was relatively flat compared with the increase in concentration but it was a clear trend and statistically significant at each concentration. After a decline at 0.5 mg DP/kg (not statistically significant at 0.1), levels of 8-OHdG actually increased with increasing concentration and were nearly back at control levels at the highest concentration of 12.5 mg/kg dw. The study authors put forward various hypotheses (involving DNA damage and repair) to explain these findings, but these remain unproven and of uncertain relevance to the viability of earthworm populations.

- AChE levels were statistically significantly inhibited at all concentrations. However, there was no concentration-dependent change and the response was again rather flat. The importance of AChE as a critical enzyme of earthworm nervous systems is unclear and whether the levels of effect seen would have caused deleterious effects is also unknown. Cellulase activity was inhibited in a similar manner at 0.5 mg/kg dw and above. The importance of cellulase activity in earthworms, and whether the level of effects seen would have adversely impacted on this, remains unclear.

- Tail DNA% (tDNA), Olive tail moment (OTM) and Tail length increased statistically significantly ($p < 0.05$) in the Comet assays for all treatments, even at the lowest test concentration of 0.1 mg/kg dw. There was no DNA damage in the control earthworms. The level of effect was not consistently concentration-related but was always greatest at the top dose. The genotoxic, biological and ecological significance of these levels of damage, given the potential for there also to be damage repair mechanisms, is unclear.

Discussion

The study was conducted (partly) in accordance with OECD TG 222, but did not investigate behaviour, feeding activity or reproductive effects. Cow manure was used as the source of organic carbon, rather than the recommended *Sphagnum* peat. As Dechlorane Plus has a high log K_{OW} and low water solubility, adsorption to high levels of organic matter in the manure substrate could potentially have affected bioavailability, which might explain the limited dose-response relationship observed for most biomarker end points. No correction for organic carbon content was undertaken. In addition, no toxic standard/reference compound (normally carbendazim or benomyl) was included, so the suitability and sensitivity of the test system used cannot be verified. OECD TG 222 also recommends at least five test concentrations and four replicates per treatment (eight for the control). Instead, this study used four test concentrations and three replicates each, including controls. Although variance in the controls and treatment groups appeared low, the relevance of this deviation to the robustness of the statistical observations is unclear.

Based on earthworm body weight and mortality, there were no statistically significant effects at any test concentration over 28 days. The 28-d LC_{50} is >12.5 mg/kg dw and the 28-d NOEC for mortality and body weight is ≥12.5 mg/kg dw.

Dechlorane Plus exposure did induce some statistically significant changes in the activities of enzyme levels potentially associated with oxidative damage. Given that some of these effects were inconsistent and not always strongly related to increasing concentration, their biological and ecological relevance is unclear. Changes in AChE activity occurred even at the lowest test concentration (0.1 mg/kg dw) and in a more concentration-dependant manner; similarly there were also statistically significant increases in indicators of DNA damage. However, the biological significance of these effect is also unclear.

The overall NOEC for these biomarker effects would be <0.1 mg/kg dw. Whilst they may suggest some potential for adverse apical effects, without clear mechanistic links, the true impact of these observations on earthworm populations remains uncertain. If there had been measurements of effects on earthworm feeding, behaviour and movement, as normally undertaken under OECD TG 222, the importance of changes such as reductions in AChE activity might have been more apparent.

Whilst the study is considered by the DS to be reliable with restrictions because it appears to have been well performed and reported (although lacking GLP), the results are of limited use due to the deviations from the standard chronic earthworm test guideline and uncertain relevance of the biomarker effects seen.

Overview of soil toxicity data

Dechlorane Plus is expected to have a high affinity for organic carbon (see Section 3.2) and soil is likely to be an important compartment for its environmental fate. The waiver put forward by the Registrant is not considered to be appropriate because it is not relevant to discount terrestrial toxicity studies based on low water solubility, there are no reliable chronic fish aquatic toxicity studies involving dietary exposure and the substance can bioaccumulate. In addition, the molecular and biomarker end points measured by Zhang *et al*. (2014) and Yang *et al*. (2014) following 14 and 28 days' exposure, respectively, imply that effects over the longer term cannot be excluded in earthworms.

Due to the lack of reliable information on Dechlorane Plus itself, Government of Canada (2016) adopted a read-across approach using chronic soil organism toxicity

test data for chlordane and mirex to screen for soil hazards. The conclusion was that Dechlorane Plus has the potential to cause effects to soil organisms such as earthworms and insects at low concentrations. However, this is a very worst case approach, especially as the analogues were pesticides (i.e. it assumes that Dechlorane Plus has the same mode of action) and the structural similarity to mirex in particular is weak (as discussed in the footnote in Section 1.4 of the main report, mirex is not a suitable analogue of Dechlorane Plus). The ECHA Read Across Assessment Framework outlines a number of steps that need to be considered (such as bioavailability, mode of action, etc.), which were not part of the Canadian assessment. Whilst acceptable for screening purposes, it is not appropriate to assume this information presents a realistic level of toxicity for Dechlorane Plus.

Further investigation of long-term terrestrial toxicity study with worms could be considered, for which an OECD TG 222: Earthworm Reproduction Test (*Eisenia fetida/Eisenia andrei*) test method would be most appropriate.

5.3 Atmospheric compartment

No data assessing effects in the atmospheric compartment are included in the registration dossier by the Registrant.

Discussion

The substance is not considered to have the potential to cause adverse effects in the atmospheric compartment because only 9×10^{-5} % to 4×10^{-3} % of Dechlorane Plus is predicted to end up in the atmosphere (see Section 3.2.3).

5.4 Microbiological activity in sewage treatment systems

5.4.1 Toxicity to aquatic micro-organisms

Toxicity to aquatic micro-organisms is waived by the Registrant based on the justification that Dechlorane Plus is highly insoluble in water and is not hazardous.

Discussion

It is not necessary to conduct a toxicity to microorganisms study based on its very low water solubility and therefore limited bioavailability. Low water solubility is a REACH Annex VIII Column 2 adaptation for this endpoint.

5.5 Non compartment specific effects relevant for the food chain (secondary poisoning)

5.5.1 Toxicity to birds

This is not an Annex IX requirement at the tonnage level of the registered substance and no standard regulatory data are presented in the registration dossier by the Registrant.

A 90-day bioaccumulation study in Common Quail (*Coturnix coturnix japonica*) by Li *et al*. (2013a) included some measures of sub-chronic toxicity (this study is also discussed in Section 3.4.2.1). A total of 60 male birds (6-8 weeks of age, with an average weight of 125 g) were acclimatised individually in suspended, unattached cages in a mass air-displacement room at 20-26 °C for 14 days. They were then randomly separated into four treatment groups (15 males/group) and orally administered the commercial substance (purity ≥ 99 % w/w) dissolved in corn oil

by gavage at nominal doses of 0, 1, 10 and 100 mg/kg bw/d for 90 days. All birds were euthanized after the experiment, and their livers weighed then subject to enzyme activity analyses (the selected enzymes were involved with xenobiotic biotransformation processes and oxidative stress) and measurement of two antioxidants (catalase (CAT) and superoxide dismutase (SOD)), glutathione and maleic dialdehyde (MDA) (an indicator of lipid peroxidation).

A NOAEL was not derived by the study authors, but no mortality or change in body or liver weight was seen following 90 days' exposure up to 100 mg/kg bw/d.

The activities of 7-ethoxyresorufin (EROD), 7-methoxyresorufin (MROD), 7-pentoxyresorufin (PROD) and 7-benzyloxy-4-trifluoromethylcoumarin debenzylase (BFCD) were lower in the 1, 10, and 100 mg/kg/d groups compared to that in the control group. The activities of PROD were significantly decreased in the exposed groups compared to the control. For hepatic microsomal erythromycin N-demethylase (ERND), the 10 and 100 mg/kg/d dosed groups showed higher enzyme activities than the control group.

The activities or levels of glutathione, CAT, SOD and MDA showed an increasing trend among the exposed groups compared to the control group. A significant increase occurred in the 10 and 100 mg/kg/d groups for CAT (p = 0.009 and 0.004, respectively) and in the 1 mg/kg/d group for SOD (p = 0.006), suggesting that Dechlorane Plus exposure may induce oxidative stress.

This study is included in the registration dossier but its validity is considered to be unassignable since it is not a classical study design and only very few sub-chronic toxicity parameters were recorded (mortality and body/liver weight). No information on behaviour or organs other than liver was provided. Observations post-exposure were not possible. In addition, rather high levels of Dechlorane Plus were detected in the control group tissues (e.g. average concentrations of the syn- and anti- isomers in muscle were 5 800 ± 2 000 and 1 000 ± 330 ng/g lw, respectively).

Crump *et al*. (2011) investigated the effects of Dechlorane Plus in Domestic Chicken (*Gallus gallus domesticus*, White Leghorn strain) embryonic hepatocytes *in vitro* and embryos following egg injection. Cultured hepatocytes were prepared from pooled liver samples taken from day-19 embryos (n=50), and exposure up to a maximum concentration of 3 µM of Dechlorane Plus took place in 48 well plates at 37 °C for 24 or 36 hours (three replicate wells per treatment group, including solvent and untreated controls), and then assessed for viability. The air space of eggs was injected with ~1 µL of either test solution (nominal concentration of 10, 100, 250 or 500 ng/g [µg/kg]) or solvent per gram of egg via a small drilled hole, which was subsequently sealed with tape (n=20 per treatment group). Pipping success was determined by dividing the number of embryos that pipped (i.e. when they start becoming active and chirping) by day 22 of incubation by the number of total fertile eggs per treatment group. Embryos that pipped successfully were euthanized and portions of the liver were removed for further analysis of gene expression (in addition, whole livers from five individuals per treatment group were randomly sampled for chemical analysis). mRNA was extracted from both embryonic hepatocytes (n=3 replicate wells for two treatment groups (i.e. 0.01 and 3 µM)) and liver (n=5–10/treatment group) for analysis of expression levels of transcripts for eleven hepatic genes previously determined to be responsive to hexabromocyclododecane (HBCDD) (related to the xenobiotic-sensing orphan nuclear receptor, the thyroid hormone pathway, lipid regulation and growth). Test solutions were prepared in dimethyl sulfoxide (DMSO), and concentrations were measured by GC-MS (ECNI).

Dechlorane Plus did not affect embryonic hepatocyte viability in comparison to solvent controls up to the highest test concentration of 3 µM (1.96 mg/L)[84]. The two treatment groups selected for mRNA isolation did not show a significant difference in viability compared to the untreated or vehicle control.

The paper reports that *in ovo* exposure did not affect pipping success since all treatment groups fell within the historical control range for DMSO-injected eggs in this laboratory (80–100 %). Nevertheless, there appeared to be a dose-dependent decrease in pipping success: 95 % in the vehicle control, 95 % at the 10 (9.1) ng/g dose, 94 % at the 100 (50) ng/g dose, 90% at the 250 (150) ng/g dose and 83 % at the 500 (191) ng/g dose (measured concentrations are given in brackets, and the difference was believed to be due to problems with dissolving the substance in DMSO). No statistical analysis was presented in the paper. Pipping success was independent of embryo sex.

Hepatic accumulation was highly variable and did not follow a linear uptake pattern based on actual injection concentration, although mean hepatic Dechlorane Plus concentrations did increase with dose group to a maximum of 85.6 ng/g ww. Liver lipid content ranged from 11 to 15 % and regardless of whether ww or lipid weight was used in the calculation, the concentration in the highest dose group was significantly greater than the 50 ng/g dose group (Dechlorane Plus was not quantifiable in livers from the 9.1 ng/g dose group). Wet weight hepatic levels were substantially lower than the measured egg injection concentrations. Hepatic enrichment factors were 0.08, 0.04 and 0.4 for the 50, 150 and 191 ng/g dose groups, respectively.

A significant shift in isomer ratio was detected between stock solutions of the commercial mixture and hepatic tissue; the proportion of the syn- isomer increased from 0.34 to 0.65 with a concomitant decrease of anti- isomer from 0.66 to 0.35. An enrichment of the syn- isomer could be explained by a number of different factors including isomer-specific bioavailability, half-life and uptake rate and/or variable depuration kinetics.

None of the investigated mRNA transcripts changed as a result of *in vitro* or *in ovo* exposure (given the lack of transcriptional response for all eleven genes following *in vitro* exposure, only a sub-set of four gene targets were included for the hepatic mRNA expression assessment: CYP3A37, DI2, L-FABP and IGF-1). The relationship between these findings and the level of Dechlorane Plus in the livers is unclear given the non-linear uptake patterns into hepatic tissue.

The study authors concluded that there were no adverse effects of Dechlorane Plus on embryonic viability or pathways associated with the genes assessed in this species. An effect on pipping success cannot be completely ruled out, but the genes identified as being sensitive to HBCDD might not be relevant to Dechlorane Plus. In addition, the embryos might not have been fully exposed to the actual administered dose (which was injected into the air space of the egg), and so the data cannot be reliably compared to concentrations observed in eggs from wild birds (in which the substance will be present within the tissues of the egg). The findings are therefore inconclusive.

This study is not included in the registration dossier.

Discussion

The available data do not suggest significant toxicity in birds, but a standard test guideline study is not available so the findings cannot be considered conclusive. Significant molecular changes were found to occur in liver following exposure of

[84] Based on the administered concentration, weight of the hepatocytes in each well and assumption of 100% uptake, this was estimated to be equivalent to 1 261 mg/kg ww.

Common Quail (*Coturnix coturnix japonica*) to a dose of 1 mg/kg bw/d for 90 days, although there was an unclear dose-response relationship. The implications of these changes are therefore also unclear. Li *et al*. (2013a) stated that they might indicate an adverse effect, since PROD activity is an indicator of CYP2B in mammals, which is, in turn, involved in the metabolism or inactivation of several endogenous chemicals such as steroid and gonadal hormones. An increased ERND activity might alter the detoxification and metabolism activities of CYP3A to endogenous and exogenous compounds. Changes in antioxidant levels suggest that Dechlorane Plus exposure may also induce oxidative stress.

5.5.2 Toxicity to mammals

See Section 4.

6 MONITORING DATA

Sverko *et al*. (2011) and Xian *et al*. (2011) discuss analytical issues.

Environmental monitoring data measured in Europe are available in the public domain, and summarised in Table 6 of Section 0 (remote regions), Appendix 3 and 4 (biota), and Appendix 5 (other compartments).

ANNEX LITERATURE SEARCH

An initial literature search was carried out on the 5th of December 2013, using available online including Chemical Abstracts and Toxnet. A combination of the following search terms were used in the search:

> 59459-11-9 or 60880-74-2 or dechlorane or 11114-14-0 or 13560-89-9 or 39386-10-2 or 40372-58-5 NOT Document Type: p AND bioaccumlat* or bioconcentrat* or toxic* or ecotox* or biodegrad* or genotox* or physicochem*

A further literature search was performed on 15 May 2015 using the abstract and citation database Scopus using the simple criteria of "Dechlorane Plus". A final literature search was undertaken on the 17 November 2015 covering the time period May 2015 to 17 November 2015. The search was carried out using PubMed using the simple criteria of "dechlorane" and "dechlorane plus". In addition a search of the non-patent literature was undertaken using Chemical Abstracts and the following combination of search terms:

> 13560-89-9 or 135821-74-8 or 135821-03-3 AND bioaccumlat* or bioconcentrat* or biomag* or magnification or toxic* or ecotox* or biodegrad* or degrad* or physicochem*.

Since the substance is sometimes included in screening studies involving many other halogenated compounds, this search strategy might not identify every study that reports data on it. The reference lists of the cited studies were therefore also checked for additional studies. Grey literature was also considered.

Given the very large number of studies published annually (often providing similar information to existing studies), no formal additional literature search has been performed since November 2015. Nevertheless, the publication of a draft Canadian screening assessment in 2016 and some more recent ecotoxicity studies have been considered as these are relevant to the T criterion. If other relevant studies are highlighted during public consultation, these can be considered in due course.

The following list of references were screened (abstracts only) for relevance, and considered to be of low importance for this report. The reason is provided after each reference.

Abbasi NA, Malik RN, Frantz A and Jaspers VL. A review on current knowledge and future prospects of organohalogen contaminants (OHCs) in Asian birds. Science of the Total Environment, 2015 Oct 29; 542(Pt A):411-426. doi: 10.1016/j.scitotenv.2015.10.088. [Epub ahead of print].
Reason for omission: Review article.

Abbasi G, Saini A, Goosey E and Diamond ML (2016). Product screening for sources of halogenated flame retardants in Canadian house and office dust. Science of the Total Environment, 545–546, 299–307.
Reason for omission: Levels in indoor products and dust from North America.

Ali U, Mahmood A, Syed JH, Li J, Zhang G, Katsoyiannis A, Jones KC and Malik RN (2015). Assessing the combined influence of TOC and black carbon in soil-air partitioning of PBDEs and DPs from the Indus River Basin, Pakistan. Environmental Pollution, 201, 131-140.
Reason for omission: Levels in soils, outside Europe.

Bao L-J, Wei Y-L, Yao Y, Ruan Q-Q and Zeng EY (2015). Global trends of research on emerging contaminants in the environment and humans: a literature assimilation. Environmental Science and Pollution Research. 22, 1635-1643.
Reason for omission: Review article only.

Cao Z, Xu F, Covaci A, Wu M, Wang H, Yu G, Wang B, Deng S, Huang J and Wang X (2014). Distribution patterns of brominated, chlorinated, and phosphorus flame retardants with particle size in indoor and outdoor dust and implications for human exposure. Environmental Science and Technology, 48 (15), 8839-8846.
Reason for omission: Levels in indoor and roadside dust, outside Europe.

Chen, S.J., Tian, M., Wang, J., Shi, T., Luo, Y., Luo, X.J., Mai, B.X., (2011). Dechlorane Plus (DP) in air and plants at an electronic waste (e-waste) site in South China. Environ. Pollut., 159, 1290–1296.
Reason for omission: Levels in air and tree bark, outside Europe.

Davis EF, Klosterhaus SL and Stapleton HM (2010). Measurement of current use flame retardant chemical in biosolids. Organohalogen Compd., 72, 1121–1224.
Reason for omission: Levels in wastewater treatment plant, outside Europe.

Davis EF, Klosterhaus and Stapleton HM (2012). Measurement of flame retardants and triclosan in municipal sewage sludge and biosolids. Environment International, 40, 1–7.
Reason for omission: Levels in wastewater treatment plant, outside Europe.

de la Torre A, Concejero MA, Sverko E, Shen L, Martinez MA, Reiner E and Alaee M (2010). Effect of source temperature on the ECNI/MS spectra of Dechlorane Plus isomers. Organohalogen Compd., 72, 1737–1740.
Reason for omission: Analytical chemistry.

Drage DS, Newton S, de Wit CA and Harrad S (2016). Concentrations of legacy and emerging flame retardants in air and soil on a transect in the UK West Midlands. Chemosphere, 148, 195-203.
Relevance: Measurements of concentrations in ambient air and soil in the UK.

Feo ML, Barón E, Eljarrat E and Barceló D (2012). Dechlorane Plus and related compounds in aquatic and terrestrial biota: a review. Anal. Bioanal. Chem., 404, 2625–2637.
Reason for omission: Review article.

Guerra P, Eljarrat E and Barceló D (2011). Determination of halogenated flame retardants by liquid chromatography coupled to mass spectrometry. TrAC Trends in Analytical Chemistry, 30, 842–855.
Reason for omission: Review article.

Hassan, Y and Shoeib T (2015). Levels of polybrominated diphenyl ethers and novel flame retardants in microenvironment dust from Egypt: An assessment of human exposure. Science of the Total Environment, 505, 47-55.
Reason for omission: Levels in indoor dust, outside Europe.

He C, Jin J, Ma Z, Wang Y, Zhaxi Z and Ma L (2013). Levels and sources of decabromodiphenyl ether and dechlorane plus in Xining and Tianjun, Qinghai Province, China. Huanjing Kexue/Environmental Science, 34(3):1129-1135.
Reason for omission: Levels in air, outside Europe.

He C, Jin J, Wang Y, Ma Z, He S and Li M (2014). Polybrominated diphenyl ethers, dechlorane plus, and polychlorinated biphenyls in tree bark near the upper Yellow River, China. Environmental Toxicology and Chemistry, 33 (8), 1732-1738.
Reason for omission: Levels in air, outside Europe.

Kakimoto K, Nagayoshi H, Akutsu K, Konishi Y, Kajimura K, Hayakawa K and Toriba A (2014). Dechlorane Plus and decabromodiphenyl ether in atmospheric particles of northeast Asian cities. Environmental Science and Pollution Research. Published on-line 16 April 2014.
Reason for omission: Levels in air, outside Europe. Similarities to BDE-209, especially in winter, was assumed to reflect a common end usage and release mechanism

La Guardia MJ, Hale RC, Harvey E and Chen D (2010). Flame-retardants and other organohalogens detected in sewage sludge by electron capture negative ion mass spectrometry. Environ Sci Technol, 44, 4658–4664.
Reason for omission: Levels in sewage sludge, outside Europe.

Lee S, Hong I-K, Ahn Y and Lee JW (2014). In-line monitoring the dispersion of highly energetic material stimulant. Polymer (Korea), 38 (3), 272-277.
Reason for omission: Polymer manufacturing techniques.

Li WL, Qi H, Ma WL, Liu LY, Zhang Z, Zhu NZ, Mohammed MO and Li YF (2015). Occurrence, behavior and human health risk assessment of dechlorane plus and related compounds in indoor dust of China. Chemosphere, 134, 166-171
Reason for omission: Indoor air levels, outside Europe.

Liu LY, Jia HL, Qi H, Ma WL, Zhang Z, Ren NQ, Cai DJ, Wan XN, Xu DD, Liu WP and Li YF (201X). Concurrently monitoring of POPs in Chinese air and surface soil - a review. Frontier of Environmental Science and Engineering, accepted for publication.
http://www.china-pops.net/yjnew_view.asp?id=822
Reason for omission: Review articles of levels in air and soil, outside EU.

Luo XJ, Chen SJ, Mai BX and Fu JM (2010). Advances in the study of current-use non-PBDE brominated flame retardants and Dechlorane Plus in the environment and humans. Sci. China Chem., 53, 961-973.
Reason for omission: Review article.

Luo P, Bao L-J, Wu F-C, Li S-M and Zeng EY (2014). Health risk characterization for resident inhalation exposure to particle-bound halogenated flame retardants in a typical e-waste recycling zone. Environmental Science and Technology, 48 (15), 8815-8822.
Reason for omission: Levels in air at e-waste recycling sites, outside Europe.

Ma WL, Liu LY, Qi H, Sun DZ, Shen JM, Wang DG and Li YF (2011). Dechlorane Plus in multimedia in northeastern Chinese urban region. Environ. Int., 37, 66–70.
Reason for omission: Levels in freshwater and soils, outside Europe.

Ma Y, Salamova A, Venier M and Hites RA (2013). Has the phase-out of PBDEs affected their atmospheric levels? Trends of PBDEs and their replacements in the Great Lakes atmosphere. Environmental Science and Technology, 47, 11457-11464.
Relevance: Levels in air and precipitation from the North American Great Lakes, outside Europe.

Ma J, Qiu X, Liu D, Zhao Y, Yang Q and Fang D (2014). Dechlorane Plus in surface soil of North China: Levels, isomer profiles, and spatial distribution. Environmental Science and Pollution Research, 21 (14), 8870-8877.
Reason for omission: Levels in soils, outside Europe.

Mahmood A, Malik RN, Li J and Zhang G (2015). Distribution, congener profile, and risk of polybrominated diphenyl ethers and dechlorane plus in water and sediment from two tributaries of the Chenab River, Pakistan. Archives of Environmental Contamination and Toxicology, 68 (1), 83-91.
Reason for omission: Levels in freshwaters and their sediments, outside Europe.

Mahmood A, Malik RN, Syed JH, Li J and Zhang G (2015). Dietary exposure and screening-level risk assessment of polybrominated diphenyl ethers (PBDEs) and dechloran plus (DP) in wheat, rice, soil and air along two tributaries of the River Chenab, Pakistan. Chemosphere, 118, 57–64.
Reason for omission: Levels in crops, soil and air, outside Europe.

Qi H, Liu L, Jia H, Li Y-F, Ren N-Q, You H, Shi X, Fan L and Ding Y (2010). Dechlorane Plus in surficial water and sediment in a northeastern Chinese river. Environ. Sci. Technol., 44, 2305–2308.
Reason for omission: Levels in freshwaters and their sediments, outside Europe.

Qiu X, Zhu T and Hu J (2010). Polybrominated diphenyl ethers (PBDEs) and other flame retardants in the atmosphere and water from Taihu Lake, East China. Chemosphere, 80, 1207–1212.
Reason for omission: Levels in air and water, outside Europe.

Ren GF, Yu ZQ, Ma ST, Chen LG, Luo XF, Sheng GY and Fu JM (2010). Dechlorane Plus in indoor and outdoor air of an urban city in southern China. Organohalogen Compd., 72, 852–855.
Reason for omission: Levels in air, outside Europe.

Salamova RA and Venier M (2010). Dechlorane Plus in Great Lakes air. Organohalogen Compd., 72, 423–426.
Reason for omission: Levels in air, outside Europe.

Salamova A and Hites RA (2010). Evaluation of tree bark as a passive atmospheric sampler for flame retardants, PCBs, and organochlorine pesticides. Environ. Sci. Technol., 44, 6196–6201.
Reason for omission: Levels in air in North America.

Salamova A and Hites RA (2011). Dechlorane Plus in the atmosphere and precipitation near the Great Lakes. Environ. Sci. Technol., 45, 9924−9930.
Reason for omission: Levels in air in the Great Lakes region of North America.

Shen KK and Sprague RW (1982). Zinc borate as a flame retardant and smoke suppressant in epoxy systems. The Journal of Fire Retardant Chemistry, 9, 161-170.
Reason for omission: Describes use as a flame retardant in epoxy resins.

Sun J, Zhang A, Fang L, Wang J and Liu W (2013). Levels and distribution of dechlorane plus and related compounds in surficial sediments of the Qiantang River in eastern China: the results of urbanization and tide. Science of the Total Environment, 443, 194-199.
Reason for omission: Levels in sediments, outside Europe.

Sverko E, Tomy GT, Marvin CH, Zaruk D, Reiner E, Helm PA, Hill B and McCarry BE (2008). Dechlorane Plus levels in sediment of the lower Great Lakes. Environ. Sci. Technol., 42, 361–366.
Reason for omission: Levels in freshwater sediments in the Great Lakes region of North America.

Venier M and Hites RA (2008). Flame retardants in the atmosphere near the Great Lakes. Environ. Sci. Technol., 42, 4745–4751.
Reason for omission: Levels in air in the Great Lakes region of North America.

Venier M, Salamova A and Hites RA (2015). Halogenated flame retardants in the Great Lakes environment. Accounts of Chemical Research, 48, 1853-1861.
Reason for omission: Review article.

Von Eyken A, Pijuan L, Martí R, Blanco MJ and Díaz-Ferrero J (2015). Determination of Dechlorane Plus and related compounds (dechlorane 602, 603 and 604) in fish and vegetable oils. Chemosphere, 144, 1256-1263.
Reason for omission: Levels in fish oil for human consumption, but unlikely to provide relevant information on whole fish concentrations.

Vorkamp K and Rigét F (2013). Nye kontaminanter med relevans for det grønlandske miljø. DCE Technical Report no. 19. http://www.dmu.dk/Pub/TR19.pdf
Reason for omission: Report is in Danish and appears to be a review of occurrence of new compounds in the Arctic. Might duplicate data in Vorkamp & Rigét (2014/5).

Wang B, Iino F, Huang J, Lu Y, Yu G and Morita M (2010). Dechlorane Plus pollution and inventory in soil of Huai'an City, China. Chemosphere, 80, 1285–1290.
Reason for omission: Levels in air and soil, outside Europe.

Wang H, Huang J, Zhang K, Yu Y, Liu K, Yu G, Deng S and Wang B (2014). Effects of zero-valent metals together with quartz sand on the mechanochemical destruction of dechlorane plus coground in a planetary ball mill. Journal of Hazardous Materials, 264, 230-235.
Reason for omission: Disposal technique.

Wang S, Wang Y, Song M, Luo C, Li J, Zhang G (2015). Distributions and compositions of old and emerging flame retardants in the rhizosphere and non-rhizosphere soil in an e-waste contaminated area of South China. Environmental Pollution, 2015 Nov 6. pii: S0269-7491(15)30139-1. doi: 10.1016/j.envpol.2015.10.038. [Epub ahead of print].
Reason for omission: Levels in soil near to a source of emission, outside Europe.

Xiang N, Chen L, Meng X-Z, Li Y-L, Liu Z, Wu B, Dai L and Dai X (2014). Polybrominated diphenyl ethers (PBDEs) and dechlorane plus (DP) in a conventional wastewater treatment plant (WWTP) in Shanghai: Seasonal variations and potential sources. Science of the Total Environment, 487 (1), 342-349.
Reason for omission: Levels in wastewater treatment plant, outside Europe.

Xu P, Tao B, Ye Z, Qi L, Ren Y, Zhou Z, Li N, Huang Y and Chen J (2015). Simultaneous determination of three alternative flame retardants (dechlorane plus, 1,2-bis(2,4,6-tribromophenoxy) ethane, and decabromodiphenyl ethane) in soils by gas chromatography-high resolution mass spectrometry. Talanta, 144, 1014-1020.
Reason for omission: Analytical method development for levels in soils, outside Europe.

Yang R, Wei H, Guo J, McLeod C, Li A and Sturchio NC (2011). Historically and currently used Dechloranes in the sediments of the Great Lakes. Environ. Sci. Technol., 45, 5156–5163.
Reason for omission: Levels in sediment, outside Europe.

Yu Z, Lu S, Gao S, Wang J, Li H, Zeng X, Sheng G and Fu J (2010). Levels and isomer profiles of Dechlorane Plus in the surface soils from e-waste recycling areas and industrial areas in south China. Environ. Pollut., 158, 2920–2925.
Reason for omission: Levels in soils, outside Europe.

Yu D, Yang J, Li T, Feng J, Xian Q and Zhu J (2015). Levels and distribution of Dechloranes in sediments of Lake Taihu, China. Environmental Science and Pollution Research, 22, 6601-6609.
Reason for omission: Levels in freshwaters and their sediments, outside Europe.

Yu Y, Hung H, Alexandrou N, Roach P and Nordin K (2015). Multiyear Measurements of Flame Retardants and Organochlorine Pesticides in Air in Canada's Western Sub-Arctic. Environmental Science and Technology, 49, 8623-8630.
Reason for omission: Levels in air, outside Europe.

Zeng L, Yang R, Zhang Q, Zhang H, Xiao K, Zhang H, Wang Y, Lam PKS and Jiang G (2014). Current levels and composition profiles of emerging halogenated flame retardants and dehalogenated products in sewage sludge from municipal wastewater treatment plants in China. Environmental Science and Technology, 48 (21), 12586-12594.
Reason for omission: Levels in wastewater treatment plant sludge, outside Europe. Statements made about stereoselective degradation based on anti-DP fractional abundance compared to the commercial substance are unreliable.

Zhang W, Huang J, Yu G, Deng S and Zhu W (2010). Mechanochemical destruction of Dechlorane Plus with calcium oxide. Chemosphere, 81, 345–350.
Reason for omission: Destruction technique.

Zhang H, Bayen S and Kelly BC (2015). Multi-residue analysis of legacy POPs and emerging organic contaminants in Singapore's coastal waters using gas chromatography-triple quadrupole tandem mass spectrometry. Science of the Total Environment, 523, 219-232.
Reason for omission: Levels in seawater, outside Europe.

Zheng Q, Nizzetto L, Li J, Mulder MD, Sáňka O, Lammel G, Bing H, Liu X, Jiang Y, Luo C and Zhang G (2015). Spatial distribution of old and emerging flame retardants in Chinese forest soils: Sources, trends and processes. Environmental Science and Technology, 49 (5), 2904-2911.
Reason for omission: Levels in soils, outside Europe.

Zheng X, Xu F, Chen K, Zeng Y, Luo X, Chen S, Mai B and Covaci A (2015). Flame retardants and organochlorines in indoor dust from several e-waste recycling sites in South China: Composition variations and implications for human exposure. Environment International, 78, 1-7.
Reason for omission: Levels in indoor dust, outside Europe.

Zhu J, Feng YL and Shoeib M (2007). Detection of Dechlorane Plus in residential indoor dust in the city of Ottawa, Canada. Environ Sci. Technol., 41, 7694-7698.
Reason for omission: Levels in indoor dust, outside Europe.

Zhu B, Lam JCW, Yang S and Lam PKS (2013). Conventional and emerging halogenated flame retardants (HFRs) in sediment of Yangtze River Delta (YRD) region, East China. Chemosphere, 93, 555–560.
Reason for omission: Levels in sediments, outside Europe.

APPENDIX 2 TRANSFORMATION PRODUCTS

A variety of substances derived from Dechlorane Plus have been detected in various monitoring studies as highlighted in Section 1.3 of the main report (e.g. Sverko *et al*., 2008 & 2010b; Muñoz-Arnanz *et al*., 2010; Möller *et al*., 2010; Guerra *et al*., 2011; Sun *et al*., 2012; Chen *et al*., 2013b; Yu *et al*., 2013; Ben *et al*., 2013 & 2014; Zheng *et al*., 2014a; Wang *et al*., 2015). There are no REACH registration dossiers for them, and reliable measured data are not available in the published scientific literature.

The two dechlorinated substances (DP-1Cl and DP-2Cl) are likely to be very hydrophobic and so their properties are probably similar to Dechlorane Plus. This appendix therefore considers other substances that may potentially be formed through transformation processes in the environment.

Sverko *et al*. (2010b) analysed a sediment core collected from the Niagara River Bar in Lake Ontario, Canada in 2007 and samples of whole Lake Trout (*Salvelinus namaycush*) (n=4) collected between 2000 and 2003 (presumably from the same location). This site is 16 km from the mouth of the Niagara River where sediment from the river first collects. Analysis following extraction was initially by GC-MS with electron capture negative ionization (ECNI) detection. Four unknown peaks were detected from the sediment core that had the same molecular weight as Dechlorane Plus. By synthesising substances for comparison, the study authors identified one vinylcyclohexene Diels-Alder reaction product that matched one of the unidentified peaks and a 1,4-cyclooctadiene Diels-Alder reaction product matching the retention time of the largest unknown peak (equivalent to a maximum concentration around one tenth the concentration of Dechlorane Plus in the same sample). 1,3-Cyclooctadiene Diels-Alder reaction products were not detected. The identity of the two remaining unknown compounds could not be confirmed, but were thought to possibly originate from 1,5-cyclooctadiene. None of these substances were detected in the Lake Trout samples.

In addition, 1,5-DPMA (Dechlorane Plus monoadduct) and tentatively 1,3-DPMA were detected in the sediment core, with amounts relative to Dechlorane Plus varying over time (presumably due to difference in the purity of starting materials). 1,3-DPMA (but not 1,5-DPMA) was detected in Lake Trout at an average concentration of 34 ± 43 µg/kg lw, which was around ten-fold higher than concentrations of Dechlorane Plus in the same samples reported by Tomy *et al*. (2007) (similar to another study by Ismail *et al*., 2009). No monoadduct compounds were detected in a technical Dechlorane Plus product (detection limit not stated).

Tomy *et al*. (2013) investigated the bioaccumulation behaviour of 1,3- and 1,5-DPMA. Levels were determined in plankton (n=1; TL=1), *Dipoeria* (n=1; TL=1.86), Alewife (n=2; TL=2.18), Smelt (n=2; TL=3.21), Sculpin (n=3; TL=3.32) and Lake Trout (n=4; TL=3.8) in a Lake Ontario food web located downstream of a Dechlorane Plus manufacturing plant. 1,3-DPMA was found in all trophic levels (detected in 92 % of samples) at concentrations between 0.12 and 199 µg/kg lipid, whereas 1,5-DPMA was detected only sporadically (38 % of samples) at concentrations up to 25.8 µg/kg lipid and was not detectable in the top predator in the food chain (Lake Trout). Tomy *et al*. (2013) noted that, where detectable, the concentrations of DPMA isomers were generally greater than the total Dechlorane Plus concentration (0.010-2.5 µg/kg lipid). The concentrations of 1,3-DPMA (and Dechlorane Plus) were generally highest in the lowest trophic levels and decreased with increasing trophic level, indicating that biodilution was occurring. 1,5-DMPA

was only detectable in two of the species (Alewife and Sculpin) and so it is not possible to identify any trends for this substance. It is important to note that the biota samples in this study were limited in number and, more importantly, were collected over a three year period (2000 to 2003). Therefore the findings in relation to biomagnification are uncertain.

Tomy *et al*. (2013) hypothesised that the prevalence of 1,3-DPMA in the food web, especially Lake Trout, may be a result of it being less readily metabolised than 1,5-DPMA. In order to test this, *in vitro* metabolism studies were carried out using Lake Trout liver microsomes. The zero-order depuration rate constants were determined to be 92.2 and 134.6 pmole h^{-1} for 1,3-DPMA and 1,5-DPMA, respectively, which supported the hypothesis that 1,5-DPMA was metabolised more readily than 1,3-DPMA.

Other studies have detected DPMA in various species of biota (see Appendix 3 and 4 for details). For example:

- Guerra *et al*. (2011) detected 1,5-DPMA in 24 Peregrine Falcon *Falco peregrinus* eggs with mean concentrations in the range 1.2 – 1 660 µg/kg lw (Great Lakes Basin, Canada) and 1.7 – 469 µg/kg lw (Bilbao, Spain) collected in the period 2003 and 2009. In contrast, it was not detected above the LoQ in White Stork *Ciconia ciconia* eggs from near Madrid (sampled in 2005, n=10) and another in the Doñana National Park (sampled in 1999–2000, n=23), Spain (Muñoz-Arnanz *et al*., 2010). The actual LoQ is not provided.

- DPMA was detected in some yellow American Eel *Anguilla rostrata* samples (whole body or muscle) collected from Lake Ontario and the Saint Lawrence River, Canada in 2007/8 by Sühring *et al*. (2014) at mean concentrations up to 0.1 µg/kg ww (0.48 µg/kg lw). It was not detected in glass eels, elvers, yellow or silver eels from France or Germany (LoD: 0.021 – 0.079 µg/kg ww).

- Wang *et al*. (2015) found DPMA to be present in a species of snail, a shrimp and five species of fish in a Chinese freshwater food chain, with mean levels between 13.3 and 65.6 µg/kg lw. The highest concentration was for Northern Snakehead *Channa argus*, a predatory fish (although sample numbers were low; these levels are much lower than the Dechlorane Plus concentrations in the same samples).

- Wolschke *et al*. (2015) detected 1,3-DPMA in tissue samples (except for blood) taken from a young Brown Skua *Stercorarius antarcticus* found dead due to natural causes on King George Island, Antarctica in the period November 2010 to January 2011. Levels were in the range 52.8–136 pg/g [ng/kg] dw. Levels in a single Antarctic Rock Cod *Trematomus bernacchii* and a young Gentoo Penguin *Pygoscelis papua* (also found dead) collected from the same area were below the detection limit. The detection frequency in the samples was 21 % for DPMA, but 7.1 % for anti-Dechlorane Plus (with a highest level of 8.9 pg/g dw). No syn-Dechlorane Plus was found in any of the investigated samples. (For comparison, see also the results of Kang *et al*. (2013) [ABST] and Kim *et al*. (2015), who detected Dechlorane Plus more frequently in similar biota samples from the same area.)

- Rjabova *et al*. (2016) detected 1,3-DPMA in muscle tissue of 25 wild Baltic Salmon *Salmo salar* collected from the Daugava and Venta rivers in Latvia during the spawning period in October 2012. It was present at a mean concentration of 969 (range 311 – 2 169) pg/g [ng/kg] ww, and contributed

49 – 76 % (average 62 %) of the total concentration of seven dechlorane-related compounds in the samples. For comparison, the mean concentration of Dechlorane Plus was 11.3 pg/g [ng/kg] ww. [This study is not summarised in Appendix 3 and 4.]

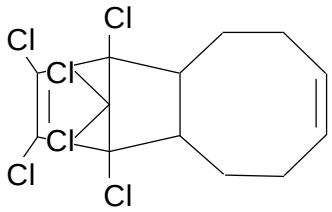
Rjabova *et al*. (2016) (citing Baron *et al*., 2012) pointed out that in order to include 1,3-DPMA in the scope of chemical analysis, the acidic clean-up step typically used for the majority of POPs has to be eliminated because of the acidic degradation of the free double bond in the monoadduct, resulting in the loss of 1,3-DPMA from the sample. Failure to use a non-destructive clean-up procedure for sample preparation could therefore lead to under-reporting.

It is therefore apparent that several substances may be present in the environment arising from the manufacture and/or degradation of Dechlorane Plus. DPMA has been detected in biota at higher concentrations that Dechlorane Plus in some samples, suggesting that it might be more bioaccumulative or is a significant transformation product. No information is available to indicate whether the levels exceed 0.1 % w/w in terms of either impurity levels or transformation.

QSAR predictions

Relevant property information for 1,5-DPMA have been estimated using *EPI Suite v4.11* (U.S. EPA, 2012). Results for 1,3-DPMA are expected to be similar as the information is derived from the structure (usually using fragments).

Structure:



SMILES code: C32(Cl)C(Cl)(Cl)C(Cl)(C(Cl)=C3Cl)C1C2CCC=CCC1
Molecular formula: $C_{13}H_{12}Cl_6$
Molecular weight: 380.96

Physico-chemical properties

Melting point: 126 °C (MPBPWIN v1.43)
Boiling point: 348 °C (Adapted Stein & Brown method, MPBPWIN v1.43)
Vapour pressure: 0.002 Pa at 25 °C (Modified Grain method, MPBPWIN v1.43)
Water solubility: 0.003 mg/L at 25 °C (Wat Sol v1.01 & WSKOW v1.42)
Log K_{OW}: 7.2 at 25 °C (KOWWIN v1.68)
Log K_{OA}: 8.5 at 25 °C (KOAWIN v1.10)

Biodegradation

Probability of Rapid Biodegradation (BIOWIN v4.10):
Biowin1 (Linear Model): -0.6539 (does not biodegrade fast)
Biowin2 (Non-Linear Model): 0.0000 (does not biodegrade fast)
Expert Survey Biodegradation Results:
Biowin3 (Ultimate Survey Model): 0.6819 (recalcitrant)
Biowin4 (Primary Survey Model): 2.2341 (months)
MITI Biodegradation Probability:
Biowin5 (MITI Linear Model): -0.0957 (does not biodegrade fast)
Biowin6 (MITI Non-Linear Model): 0.0000 (does not biodegrade fast)
Anaerobic Biodegradation Probability:

Biowin7 (Anaerobic Linear Model): -0.8028 (does not biodegrade fast)
Ready Biodegradability Prediction: NO

Bioaccumulation
For comparison with the REACH criteria, EA (2013) recommends that the BCF estimate from the regression equation is preferred over the lower trophic level estimates from the Arnot-Gobas model (upper trophic level estimates are not recommended to be used).

BCFBAF v3.01, using estimated log K_{OW}
BCF (regression-based method) = 11 140 L/kg ww
Biotransformation half-life normalised to a 10 g fish: 162 days[85]
BCF Arnot-Gobas method (lower trophic) = 10 580 L/kg ww (assuming biotransformation)
BAF Arnot-Gobas method (lower trophic) = 1 684 000 L/kg ww (assuming biotransformation)

N.B. The BCFBAF program estimates the BCF (and BAF) on the basis of the total concentration in water rather than the dissolved concentration in water, so a BCF based on dissolved concentrations may be higher than suggested here.

Aquatic toxicity

ECOSAR Class Program (ECOSAR v1.11):
Vinyl/allyl halides model:

Fish 96-h LC_{50}:	0.0015 mg/L
Daphnid 48-h LC_{50}:	0.0017 mg/L
Green algae 96-h EC_{50}:	0.011 mg/L*
Fish ChV:	*0.00002 mg/L*
Daphnid ChV:	*0.008 mg/L**

[Green algae ChV not reported due to weak training set – for reasons, see Appendix 8]

Neutral organics model:

Fish 96-h LC_{50}:	0.007 mg/L*
Daphnid 48-h LC_{50}:	0.007 mg/L*
Green algae 96-h EC_{50}:	0.036 mg/L*
Fish ChV:	*0.0012 mg/L*
Daphnid ChV:	*0.002 mg/L*
Green algae ChV:	0.027 mg/L*

* Indicates that the chemical may not be soluble enough to measure this predicted effect.

ChV (Chronic Value) is the geometric mean of the no observed effect concentration (NOEC) and the lowest observed effect concentration (LOEC). This can be mathematically represented as: ChV = 10^([log (LOEC x NOEC)]/2). NOEC values can be derived from predicted ChV values by dividing by √2.

The reliability of the EPIWIN ecotoxicity QSAR models for this type of structure are discussed in Appendix 8. DPMA has a predicted log K_{OW} that is outside of the prediction domain for the acute toxicity models, so the acute predictions may be

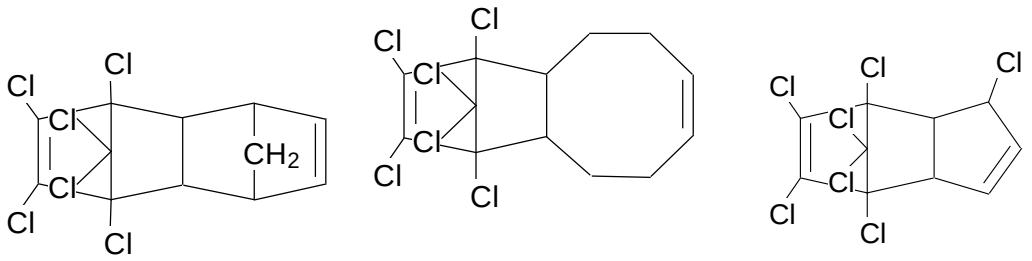
[85] Sverko *et al*. (2010c) [ABST] determined metabolic half-lives of 2.5 and 1.1 h for 1,3-DPMA and 1,5-DPMA, respectively, using *in vitro* liver microsomes from Lake Trout.

unreliable. The log K_{OW} is below 8 so within the prediction domain of the chronic models. The algal predictions are higher than the predicted water solubility by an order of magnitude, so long-term effects are not expected. There is one relevant analogue (chlordane) in the training set for the chronic fish/daphnia QSAR in the vinyl/allyl halides model. There are no norbornene analogues in the training set for the chronic QSAR in the neutral organics model. It is concluded that DPMA might be toxic to fish and Daphnia with a chronic NOEC below 0.01 mg/L, although there is some uncertainty. These QSAR estimates might not take full account of specific modes of action.

Read across

DPMA has structural similarities to aldrin (CAS no. 309-00-2) and heptachlor (CAS no. 76-44-8), both well-known pesticide active substances and Persistent Organic Pollutants (see Section 1.4 of the main report).

Aldrin **DPMA** **Heptachlor**



All share the chlorinated norbornene group attached to an aliphatic ring containing one double bond. DPMA's ring is eight-sided, whereas aldrin's is six-sided and also has an aliphatic bridge, which creates rigidity (DPMA will have some limited conformational flexibility). Heptachlor has a five-membered aliphatic ring with a chlorine atom and also has a fixed conformation.

The molecular formulas and masses are similar ($C_{13}H_{12}Cl_6$, 380.96 for DPMA, $C_{12}H_8Cl_6$, 364.92 for aldrin and $C_{10}H_5Cl_7$, 373.32 for heptachlor). Molecular dimensions are also broadly similar. Using the OECD QSAR Toolbox v2.1, the following parameters can be estimated:

Property		**DPMA**	**Aldrin**	**Heptachlor**
Molecular diameter, Å	Maximum	11.2	9.9	9.7
	Minimum	7.3	7.3	7.6
	Effective	8.7	8.7	8.6
van der Waal's volume, $Å^3$		231	210	202

DPMA might be less bioavailable: water solubilities and log K_{OW} values are 0.003 mg/L and 7.2 (predicted) for DPMA, 0.017 mg/L and 6.5 (measured) for aldrin and 0.18 mg/L and 5.5-6.1 (measured) for heptachlor (see Table 1 in the main report).

WHO (1989) reports that aldrin is rapidly converted to dieldrin by epoxidation of the double bond in the environment, and that a large number of microorganisms are capable of promoting epoxidation. Aldrin and dieldrin are highly toxic substances[86], and dieldrin is much more resistant to biodegradation (WHO, 1989).

[86] Aldrin has a harmonised hazard classification under the CLP Regulation as: Acute Tox. 3 (H301 & H311), Carc. 2, STOT RE 1 (H372), Aquatic Acute 1 and Aquatic Chronic 1. Dieldrin has a harmonised

Their historical use as neurotoxic insecticides means that they target the central nervous system, but a variety of other effects have been observed in mammals and birds, including on the immune system and liver (WHO, 1989). The mode of action could involve the alkyl bridge (e.g. via hydroxylation), so this does not automatically mean that DPMA would cause effects of the same type or at similar concentrations.

Heptachlor is also highly toxic[87]. EFSA (2007) highlights that the two main transformation products of heptachlor – heptachlor epoxide and photoheptachlor – are persistent, lipophilic and toxic.

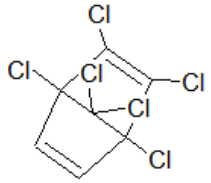
If DPMA is similarly converted to an epoxide in the environment, the resulting substance could be highly persistent, bioaccumulative and toxic to both invertebrates and vertebrates, by analogy with dieldrin and heptachlor epoxide. Experimental data would be needed to verify this.

Other potential transformation products

Hexachloronorbornadiene is a potential impurity or degradation product of chlorinated cyclodiene pesticides such as dieldrin, aldrin, etc. It is also a potential counterpart in the reaction that leads to the formation of 1,5-DPMA. The US EPA (1977) performed a 30-d toxicity test with larval-early juvenile Fathead Minnows *P. promelas* using a flow-through system (the same study appears to be reported as Spehar *et al*., 1979). It was conducted at 25 °C and pH 7.2 – 7.7 (other water quality parameters were also monitored and reported). Five test concentrations were prepared using acetone as a solvent (at 4 mg/L in the final test solutions). Nominal concentrations are not stated but the measured concentrations ranged from <0.04 (control) to 226 µg/L [0.226 mg/L]. Significant mortality occurred at 0.122 and 0.226 mg/L after 4 days. Growth was the most sensitive end point and was significantly reduced at 0.0384 mg/L; the 30-d $NOEC_{growth}$ was 0.02 mg/L (measured concentration). Fish concentrations were also measured at the end of the test and the BCF was 6 400 L/kg at the level of the NOEC (no information is available about lipid content, or whether steady state had been reached). The study was performed in a government laboratory but the method pre-dates OECD TG 210, and so is not directly comparable with the long-term fish tests currently recommended. Results for hexachlorocyclopentadiene are included in the same report, and the study was considered reliable in the EU evaluation of that substance (EC, undated). EC (undated) noted that invertebrates (mysid shrimp) were about an order of magnitude more sensitive to hexachlorocyclopentadiene than fish in long-term tests, so the same may be true of hexachloronorbornadiene.

Relevant property information for hexachloronorbornadiene have been estimated using *EPI Suite v4.11* (U.S. EPA, 2012).

Structure:



hazard classification under the CLP Regulation as: Acute Tox. 3 (H301), Acute Tox. 1 (H310), Carc. 2, STOT RE 1 (H372), Aquatic Acute 1 and Aquatic Chronic 1.

[87] Heptachlor has a harmonised hazard classification under the CLP Regulation as: Acute Tox. 3 (H311), Carc. 2, STOT RE 2 (H373), Aquatic Acute 1 and Aquatic Chronic 1.

SMILES code: ClC1=C(Cl)C2(Cl)(C=CC1(Cl)C2(Cl)(Cl))
IUPAC name: 1,2,3,4,7,7-Hexachlorobicyclo[2.2.1]hepta-2,5-diene
CAS no.: 3389-71-7
EC no.: 222-220-8
Molecular formula: $C_7H_2Cl_6$
Molecular weight: 295.83

Physico-chemical properties

Melting point: 79 °C (MPBPWIN v1.43)
Boiling point: 261 °C (Adapted Stein & Brown method, MPBPWIN v1.43)
Vapour pressure: 0.62 Pa at 25 °C (Modified Grain method, MPBPWIN v1.43)
Water solubility: 0.5 mg/L at 25 °C (Wat Sol v1.01 & WSKOW v1.42)
Log K_{OW}: 5.15 at 25 °C (KOWWIN v1.68)
Log K_{OA}: 6.8 at 25 °C (KOAWIN v1.10)

Biodegradation

Probability of Rapid Biodegradation (BIOWIN v4.10):
Biowin1 (Linear Model): -0.6148 (does not biodegrade fast)
Biowin2 (Non-Linear Model): 0.0000 (does not biodegrade fast)
Expert Survey Biodegradation Results:
Biowin3 (Ultimate Survey Model): 0.8634 (recalcitrant)
Biowin4 (Primary Survey Model): 2.3526 (weeks-months)
MITI Biodegradation Probability:
Biowin5 (MITI Linear Model): 0.0449 (does not biodegrade fast)
Biowin6 (MITI Non-Linear Model): 0.0002 (does not biodegrade fast)
Anaerobic Biodegradation Probability:
Biowin7 (Anaerobic Linear Model): -0.4016 (does not biodegrade fast)
Ready Biodegradability Prediction: NO

Bioaccumulation
BCFBAF v3.01, using estimated log K_{OW}
BCF (regression-based method) = 1 169 L/kg ww
Biotransformation half-life normalised to a 10 g fish: 25 days
BCF Arnot-Gobas method (lower trophic) = 4 973 L/kg ww (assuming biotransformation)
BAF Arnot-Gobas method (lower trophic) = 13 000 L/kg ww (assuming biotransformation)

N.B. The BCFBAF program estimates the BCF (and BAF) on the basis of the total concentration in water rather than the dissolved concentration in water, so a BCF based on dissolved concentrations may be higher than suggested here. The prediction using the Arnot-Gobas method seems to support the BCF above 5 000 L/kg reported by US EPA (1977) in a non-standard test (see above).

Aquatic toxicity

ECOSAR Class Program (ECOSAR v1.11):
Vinyl/allyl halides model:

Fish 96-h LC_{50}:	0.079 mg/L
Daphnid 48-h LC_{50}:	0.079 mg/L
Green algae 96-h EC_{50}:	0.28 mg/L
Fish ChV:	*0.004 mg/L*
Daphnid ChV:	*0.027 mg/L*

[Green algae ChV not reported due to weak training set – for reasons, see Appendix 8]

Neutral organics model:

Fish 96-h LC_{50}:	0.36 mg/L
Daphnid 48-h LC_{50}:	0.27 mg/L
Green algae 96-h EC_{50}:	0.68 mg/L*
Fish ChV:	*0.05 mg/L*
Daphnid ChV:	*0.06 mg/L*
Green algae ChV:	0.34 mg/L

* Indicates that the chemical may not be soluble enough to measure this predicted effect.

The reliability of the EPIWIN ecotoxicity QSAR models are discussed in Appendix 8. Hexachloronorbornadiene has a predicted log K_{OW} that is within the prediction domain of all of the QSARs except the acute fish and invertebrate QSARs in the neutral organics model, which it marginally exceeds (upper limit 5.0). There is one relevant analogue (heptachlor) in the training set of the acute fish/daphnia QSAR and one other (chlordane) in the training set for the chronic fish/daphnia QSAR in the vinyl/allyl halides model. There are no norbornene analogues in the training set for the neutral organics model. It can be concluded that hexachloronorbornadiene might be toxic to fish and Daphnia with a chronic NOEC in the range 0.001 – 0.1 mg/L. This is consistent with the reported 30-d $NOEC_{growth}$ for fish of 0.02 mg/L from a non-standard test (US EPA, 1977).

There is one aggregated notification on the CLP Inventory for this substance, with a self-classification of Aquatic Acute 1 and Aquatic Chronic 1 and indication that data is lacking for all other classification end points.

Conclusion

Whilst there is uncertainty in the QSAR predictions, especially given the limited number of relevant analogues in the training sets, 1,3-/1,5-DPMA screens as being potentially (very) persistent and very bioaccumulative, and is also potentially toxic based on chronic toxicity predictions for fish and aquatic invertebrates (i.e. it meets the screening PBT and/or vPvB criteria of REACH Annex XIII). No information is available on mammalian toxicity, but if it reacts like aldrin or heptachlor to form an epoxide in the environment, it could be neurotoxic and/or cause hepatotoxicity.

Hexachloronorbornadiene (if formed) screens as potentially (very) persistent and very bioaccumulative (based on a non-standard fish test result and the Arnot-Gobas estimate) and potentially T (based on aquatic ecotoxicity predictions for fish and invertebrates) (i.e. it might meet the PBT and/or vPvB criteria of REACH Annex XIII). The 30-d NOEC of 0.02 mg/L from a non-standard long-term fish toxicity study performed in the 1970s does not trigger the T criterion, although it did not

include all relevant end points that would be reported in a modern test. The level of toxicity to mammals is unknown.

A definitive conclusion would require experimental data to confirm that these degradants are formed in relevant amounts in standard transformation studies, and also to confirm their properties.

APPENDIX 3 MEASURED CONCENTRATIONS OF DECHLORANE PLUS IN BIOTA ASSOCIATED WITH THE AQUATIC ENVIRONMENT

Species	Tissue	Sample location and date	Limit of detection (LoD), µg/kg ww	Measured concentration	Comment	Reference
INVERTEBRATES						
Mussel (species not specified)	Soft parts	Niagara River area, Canada (it appears that two different locations were involved, with one mussel representing each site); date of sampling not stated	-	Site 1 (n=1) Total isomers: ~4 µg/kg ww Anti-DP: ~2 µg/kg ww Syn-DP: ~2 µg/kg ww Site 2 (n=1) Total isomers: ~1.8 µg/kg ww Anti-DP: ~1 µg/kg ww Syn-DP: ~0.8 µg/kg ww	Analysis by GC-HRMS. Not known if mussels were depurated prior to analysis. Values read from a graph.	Kolic *et al*. (2009)
Blue Mussel (*Mytilus edulis*)	Soft parts	Receiving water from Åse WWTP, Åsefjorden, Norway (sampling date not stated). Fossá river estuary, Hvalfjörður, Iceland in November 2011.	Not explicitly stated, but presumably 0.003/4 for both isomers	Norwegian (urban) site: Total isomers: 0.035-0.042 µg/kg ww Anti-DP: 0.018-0.019 µg/kg ww Syn-DP: 0.017- 0.023 µg/kg ww Icelandic site: Neither isomer was above the LoD (no. of samples not stated).	Analysis by GC-MS. Not known if mussels were depurated prior to analysis. The Icelandic site is remote from human activity.	Schlabach *et al*. (2011)

Species	Tissue	Sample location and date	Limit of detection (LoD), µg/kg ww	Measured concentration	Comment	Reference
FISH						
Barbel (*Barbus barbus*) (n not stated) Wels Catfish (*Silurus glanis*) (n not stated) Common Carp (*Cyprinus carpio*) (n not stated)	Not stated	Ebro river basin, Spain (year not stated)	Anti-DP: 0.0023 Syn-DP: 0.0055 (µg/kg lw)	Total isomer concentration: Median: 0.88 µg/kg lw Range: <LoD – 2.24 µg/kg lw	Analysis by GC-NCI-MS-MS. Presumably a sub-set of the data reported by Santín *et al*. (2013).	Barón *et al*. (2012)
Fish (various species) (n=48)	Whole body	Llobregat, Ebro, Júcar and Guadalquivir river basins, Spain; 2010	Anti-DP: 0.0023 Syn-DP: 0.0055 (µg/kg lw)	Concentration ranges (total isomers), µg/kg lw: Llobregat river basin: 0.57–4.86 Ebro river basin: 0.11–1.28 Júcar river basin: <LoD-0.59 Guadalquivir river basin: 0.06–1.91	Analysis by GC-MS. The study included four Wels Catfish (*Silurus glanis*) caught in the Ebro river basin.	Santín *et al*. (2013)
Lake Trout (*Salvelinus namaycush*) Whitefish (*Coregonus clupeaformis*)	Dorsal muscle	Lake Trout from Lake Superior in 2002 (n=3), Lake Huron in 2001 (n=5) and Lake Ontario in 1998 and 1999 (n=5), Canada. Whitefish from Lake Erie in 2002 (n=5) and Lake Ontario in 2000 (n=5).	-	Detected in all samples in the range 0.061 – 2.600 µg/kg lw (total isomers) Fish from Lake Ontario had higher concentrations compared to those from the other lakes.	Analysis by GC-HRMS. Most fish samples had f_{anti} values below the highest value of technical products (no difference was observed between the two fish species).	Shen *et al*. (2010)

Species	Tissue	Sample location and date	Limit of detection (LoD), µg/kg ww	Measured concentration	Comment	Reference
Walleye (*Stizostedion vitreum)*	?	Lake Erie, USA; 1980, 1984, 1990, 1992, 1994, 1996, and 2000. Except for 1980 and 1984, there were three samples in each sampling year.	-	Range: 0.14-0.91 µg/kg lw	Analysis by GC-MS.	Hoh *et al*. (2006)
Yellow Perch (*Perca flavescens*) (n = 29)	Whole body	St. Lawrence River and tributaries, Canada; 2008–2012	Anti-DP: 0.05 Syn-DP: 0.12 (LoQ, µg/kg lw)	Not detected in any sample	Analysis by GC-MS. Most of the Muskelunge samples were >7 years old.	Houde *et al*. (2014)
Northern Pike (*Esox lucius*) (n=11)	Liver			Detected in 45 % of samples. Range: not detected to 9.1 (syn-) or 2 (anti-) µg/kg lw		
Muskellunge (*Esox muskellunge*) (n=10)	Liver			Detected in at least 90% of samples. Mean concentration (total isomers): 6.2 ± 3.6 µg/kg lw (one fish contained 37.4 µg/kg lw)		
Lake Trout (*Salvelinus namaycush*) (n=5 per year)	Whole body	Lake Ontario (north of Main Duck Island), Canada; 1979, 1983, 1988, 1993, 1998 & 2004	0.01	Mean per year (total isomers): 0.31±0.07 to 0.85±0.20 µg/kg ww [2.3±0.6 to 7.2±1.3 µg/kg lw]	Analysis by GC-MS. Sampled fish were four to five years old. Stable isotope analysis showed that trophic status and food sources were highly variable over time.	Ismail *et al*. (2009)

Species	Tissue	Sample location and date	Limit of detection (LoD), µg/kg ww	Measured concentration	Comment	Reference
European Eel (*Anguilla anguilla*)	Whole body or muscle	Glass eels (n=100, split into 10 samples) collected from the French Atlantic coast; date not stated Elvers (n=30), yellow (n=30) and silver eels (n=12) collected from Germany (River Vidå, River Elbe and Rivers Elbe and Rhine, respectively); date not stated	Anti-DP: 0.017 Syn-DP: 0.0053	Detected in all life stages. Total isomer concentrations were: Glass eels: <0.02 – 0.32 µg/kg ww [<LoD – 31.8 µg/kg lw] Elvers: <0.02 – 0.46 µg/kg ww [<LoD – 33.8 µg/kg lw] Yellow eels: 0.013–0.50 µg/kg ww [0.14±0.008 µg/kg lw] Silver eels: 0.017–0.38 µg/kg ww [0.17±0.19 µg/kg lw]	Analysis by GC-MS. Levels were similar to American Eels, and probably reflect diffuse exposure. The isomer ratio changes over the life cycle. The syn- isomer predominates (>80%) in glass, elvers and yellow eels, but its contribution drops to 40% in silver (fully adult) eels that have stopped feeding.	Sühring *et al*. (2013 and 2014)

Species	Tissue	Sample location and date	Limit of detection (LoD), µg/kg ww	Measured concentration	Comment	Reference
American Eel (*Anguilla rostrata*)	Whole body or muscle	Glass eels (n=37, pooled into three samples) from Baie des Sables, Matane, Quebec, Canada; 2007 and 2008 Young yellow eels (n=10) from the Saint Lawrence River, Canada; 2007 and 2008 Yellow eels (n=15, muscle) from Lake Ontario and the upper Saint Lawrence River, Canada; 2007 and 2008 Silver eels from Lake Ontario, Canada; 2007 and 2008	Anti-DP: 0.017 Syn-DP: 0.0053	Detected in all life stages. Total isomer concentrations were: Glass eels: <0.02 µg/kg ww Young yellow eels: 0.10–0.69 µg/kg ww [1.7 ± 0.92 µg/kg lw] Yellow eels: 0.19 ± 0.086 to 0.29 ± 0.20 µg/kg ww [0.90 ± 0.41 to 0.17±0.19 µg/kg lw] Silver eels: 0.067 ± 0.048 µg/kg lw DPMA was detected in yellow and silver eels from the same area.	Analysis by GC-MS. Levels were similar to European Eels, and probably reflect point source as well as diffuse exposure. The isomer ratio changes over the life cycle. The syn- isomer predominates (>70%) in yellow eels, but its contribution drops to 44 % in silver (fully adult) eels that have stopped feeding.	Sühring *et al*. (2014) & Byer *et al*. (2013)

Species	Tissue	Sample location and date	Limit of detection (LoD), µg/kg ww	Measured concentration	Comment	Reference
Perch (*Perca fluviatilis*)	Muscle	One composite sample from Helsinki (Old City Bay) and five composite samples from Pyhäjärvi, Tampere. Finland (6–10 individuals per composite) (sampling date not stated). Two locations (Riddarfjärden and Stora Essingen) at Lake Mälaren, Stockholm, Sweden (sampling date not stated).	Anti-DP: 0.001-0.003 Syn-DP: 0.002-0.004	Finnish sites: Anti-DP: 0.0011 & 0.0030 µg/kg ww in two composite samples, all others below LoD Syn-DP: 0.0038 µg/kg ww in one composite sample, all others below LoD. Swedish site: Neither isomer was above the LoD (no. of samples not stated).	Analysis by GC-MS. The sites are in urban areas.	Schlabach *et al*. (2011)
Arctic Char (*Salvelinus alpinus*)	Muscle	á Mýranar lake, Faroe Islands (sampling date not stated).	Not explicitly stated, but presumably 0.003/4 for both isomers	12 fish analysed as a pooled sample. Neither isomer was above the LoD.	Analysis by GC-MS. Site is remote from human activity.	Schlabach *et al*. (2011)

Species	Tissue	Sample location and date	Limit of detection (LoD), µg/kg ww	Measured concentration	Comment	Reference
Striped Bass (*Morone saxatilis*) (n=1) Tilapia (*Oreochromis mossambicus*)] (n=1) Cod (*Gadus morhua*) (n=1) Atlantic Salmon (*Salmo salar*)] (n=1)	Muscle	Two Supermarket in Chung-Li city, Taiwan (the cod and salmon were imported, the other two species were locally caught).	0.0003 µg/g lw (both isomers)	Anti-DP: range 0.034 – 0.300 µg/kg lw Syn-DP: range 0.038 – 0.273 µg/kg lw The highest concentrations occurred in the bass.	Analysis was by GC-MS.	Chen *et al*. (2014)

Species	Tissue	Sample location and date	Limit of detection (LoD), µg/kg ww	Measured concentration	Comment	Reference
Fish (15 marine species) (n=20)	Muscle	Supermarkets in Osaka, Japan; June 2011	0.0002	Detected in 18 out of 20 samples, up to 0.0142 µg/g ww	Analysis was by GC-MS.	Kakimoto *et al*. (2012)
Common Mullet Oriental Goby Steed Barbel Temperate Sea Bass Crucian Carp (Latin names not provided)	Muscle	22 rivers across South Korea from late July to early October 2008. Urban-industrial: 15 sites Rural-industrial: 3 sites Rural: 4 sites Fish were sampled twice at each site, and several individual fish carcasses were combined and homogenized to provide a pooled sample.	Not stated	Average total concentration: Overall: 24.5 (range: 0.61 – 126) µg/kg lw Urban-industrial region: 36.1±35.3 ng/g lw Rural region: 1.4±1.0 µg/kg lw Concentrations of syn- and anti-DP isomer ranged from 0.17 – 30 µg/kg lw and 0.44 – 97 µg/kg lw, respectively. The anti-DP isomer was dominant in all samples. The mean f_{anti} value (0.67 ±0.060) was significantly lower than that of the technical product (0.75) (p = 0.032) suggesting that the syn- isomer may be more bioaccumulative.	Analysis was by GC- high resolution MS. Both isomers were consistently detected in all fish samples regardless of sampling sites and fish species. Mean concentrations at the urban sites were around 25 times greater than those at the rural sites. There is no manufacturing facility in South Korea.	Kang *et al*. (2010); Kang *et al*. (2009) [ABST].
Mud Carp (*Cirrhinus molitorella*) (n=10)	Muscle, liver & brain	Natural pond at an e-waste recycling site, South China; December 2009	Anti-DP: 0.00052 (muscle) to 0.024 (brain)	Both isomers were detected in all samples. Median concentrations of total isomers were as follows:	Analysis was by GC-MS. Both species are associated with benthic	Zhang *et al*. (2011a)

Northern Snakehead (*Channa argus*) (n=10)			Syn-DP: 0.0012 (muscle) to 0.055 (brain)	Mud Carp Muscle: 0.38 µg/kg ww Liver: 9.55 µg/kg ww Brain: 18.26 µg/kg ww Northern Snakehead Muscle: 0.76 µg/kg ww Liver: 92.0 µg/kg ww Brain: 11.8 µg/kg ww Anti-DP-1Cl was detected in 100% of liver and 80% of muscle samples, with median concentrations of 0.01-5.63 µg/kg ww. Anti-DP-2Cl was detected in one muscle sample of Mud Carp, at a concentration of 0.01 µg/kg ww. Both isomers were detected in all five sediment samples collected at the same time (at concentrations from 6.32 to 25.0 (median: 12.0) µg/kg and from 0.42 to 0.83 (median: 0.64) µg/kg dw, respectively).	environments. The median sediment concentration (total isomers) was above 3,000 µg/kg dw. Higher levels of the anti-isomer were detected in the brain than liver or muscle for Mud Carp, whereas liver accumulated more of both isomers in Northern Snakehead. Lipid-normalized concentrations indicated preferential distribution to liver in both species, suggesting that hepatic proteins might be important in the accumulation of this substance. It appeared that there was enrichment of the syn-isomer in all tissues (except Northern Snakehead brain) compared to levels in sediment and the technical product. The study shows that both isomers can cross the blood-brain barrier in fish.	

Species	Tissue	Sample location and date	Limit of detection (LoD), µg/kg ww	Measured concentration	Comment	Reference
Mud Carp (*Cirrhinus molitorella*) Northern Snakehead (*Ophicephalus argus*)	Blood serum	Electronics waste recycling site in south China, 2010. 3 pooled samples for each species each taken from 6 individuals. Each pooled sample was divided into 2 subsamples for analysis	0.009-0.026	Detected in all pooled samples Mud Carp Total isomers: Mean: 0.3 µg/kg ww Mean f_{anti} = 0.44 Northern Snakehead Total isomers: Mean:4.6 µg/kg ww Mean f_{anti} = 0.56	Analysis by GC-MS. The range of concentrations was given as 0.3-5.1 µg/kg ww or 47-727 µg/kg lw. The lipid weight concentrations are not given separately for each species. The f_{anti} in both species was significantly lower (p<0.001) than that in sediments from the area (f_{anti}=0.755).	Zeng *et al.* (2014b)
Crucian Carp (*Carassius carassius*) Common Carp (*Cyprinus carpio*) Grass Carp (*Ctenopharyngodon idellus*) Sharpbelly (*Hemiculter leucisculus*) Pond Loach (*Misgurnus anguillicaudatus*)	Muscle	Liaohe River, Liaoning province, China; August 2010. 18 pooled samples (6 sites)	-	Detected in 17 out of 18 pooled samples Total isomers: Mean: 223 ng/kg lw Median: 215 ng/kg lw Range: not detected – 470 ng/kg lw	Analysis by GC-MS.	Ren *et al*. (2013)

Species	Tissue	Sample location and date	Limit of detection (LoD), µg/kg ww	Measured concentration	Comment	Reference
Bleeker (*Pseudolaubuca sinensis*) (n = 12) Loach (*Misgurnus anguillicaudatus*) (n = 7) Crucian Carp (*Carassius auratus*) (n = 9) Common Carp (*Cyprinus carpio*) (n = 8) Northern Snakehead (*Channa argus*) (n = 3)	Muscle	Beijing–Hangzhou Grand Canal, Huai'an, Jiangsu province, China; May 2010. Five fish were pooled into composite samples for each species (except Northern Snakehead).	Anti-DP: 0.135 Syn-DP: 0.120	Total isomers: Mean: 764 (range of means for each species: 56.8 to 1 110) µg/kg ww Mean: 67 500 µg/kg lw (range of means for each species: 2 760−96 800 µg/kg lw)	Analysis by GC- high resolution MS. The site was downstream of the discharge point of the Chinese manufacturing facility. The highest mean concentration was 1.1 mg/kg ww in Common Carp, or 97 mg/kg lw in Bleeker.	Wang *et al*. (2013)

Species	Tissue	Sample location and date	Limit of detection (LoD), µg/kg ww	Measured concentration	Comment	Reference
Mosquito Fish (*Gambusia affinis*) (n = 11) Paradise Fish (*Macropodus opercularis*) (n = 9) Chinese Hooksnout Carp (*Opsariichthys bidens*) (n = 18). Chinese False Gudgeon (*Abbottina rivularis*) (n = 10) Nichols' Minnow (*Nicholsicypris normalis*) (n = 6) Chinese Bitterling (*Rhodeinae*) (n = 9)	Whole fish	E-waste recycling site & reference site (Dinghu Mountain) in the Pearl River Delta, Guangdong Province, southern China; March - July 2010. Fish were pooled into composites for each species at each site.	Anti-DP: 0.59 (lw) Syn-DP: 0.14 (lw)	E-waste recycling site (29 fish, 9 composites) Total isomers: medians per species: 79-410 µg/kg lw (overall range 60-420 µg/kg lw) Reference site (34 fish, 12 composites) Total isomers: medians per species: 1.7-8.4 µg/kg lw (overall range 0.96-8.8 µg/kg lw) Anti-DP-1Cl was detected in all samples collected from the e-waste recycling site (range: 2.4-14 µg/kg lw), but not at the reference site (LoD 0.09 µg/kg lw). Anti-DP-2Cl was not detected in any sample (LoD 0.01 µg/kg lw).	Analysis by GC-MS. The e-waste site is in a heavily industrialized area. The reference site is in a relatively non-contaminated agricultural area.	Mo *et al*. (2013)

Species	Tissue	Sample location and date	Limit of detection (LoD), µg/kg ww	Measured concentration	Comment	Reference
Greenland Shark (*Somniosus microcephalus*)	Liver	Female sharks by-caught in a commercial fishery in the waters around Iceland, northeast Atlantic, between April 2001 and October 2003 (n=15).	Not stated	Not stated.	The paper provides quantitative data on three target compounds that were "routinely" detected in the liver samples, but does not comment on the concentrations or detection frequencies of the other substances that were included in the analysis (including Dechlorane Plus). In summarising this study, Vorkamp & Rigét (2014) stated that Dechlorane Plus was "not detected", but this might be misleading.	Strid *et al*. (2013)

Species	Tissue	Sample location and date	Limit of detection (LoD), µg/kg ww	Measured concentration	Comment	Reference
Brown trout (*Salmo trutta*)	Fillet	One location in Lake Mjøsa, Norway, August 2016.	Anti-DP: 0.14 Syn-DP: 0.03	Not detected in 10 fillet samples	Number of fish not specified	Schlabach *et al*. (2017)

Species	Tissue	Sample location and date	Limit of detection (LoD), µg/kg ww	Measured concentration	Comment	Reference
BIRDS						
Bald Eagle (*Haliaeetus leucocephalus*)	Blood serum	Great Lakes region, North America; nestlings sampled from early May to late June of 2005	Not stated	Detected in 6 samples out of 12. Average (total isomers): 0.19 ± 0.10 µg/kg ww	Analysis by GC-MS.	Venier *et al*. (2010)

Species	Tissue	Sample location and date	Limit of detection (LoD), µg/kg ww	Measured concentration	Comment	Reference
Peregrine Falcon (*Falco peregrinus*)	Eggs	Great Lakes Basin, Canada (Lake Superior, Lake Ontario and the St. Lawrence River, and near St. John, New Brunswick); between 2007 and 2009 (n=2) Bilbao, Spain; from 2003 to 2006 (n=8)	0.012 µg/kg lw	Dechlorane Plus was detected in all of the samples (n=10). Geometric means with ranges are: Canada 27.7 (6.3 & 122) µg/kg lw Spain 2.8 (0.4 – 17) µg/kg lw Anti-DP-1Cl and anti-DP-2Cl were detected in both eggs from Canada, at concentrations in the range 1.6 to 2.4 µg/kg lw. However, in the Spanish samples, anti-DP-1Cl was only detected in six eggs (in the range not quantifiable – 0.28 µg/kg lw), and anti-DP-2Cl was not detected. 1,5-DPMA was detected in 9 eggs with concentrations in the range 3.8 – 218 µg/kg lw (Canada) and 18 – 469 µg/kg lw (Spain).	Analysis was by GC-HRMS. The Canadian results are thought to reflect local exposure based on proximity to one of the known production sites. Canadian food chains were determined by prey analysis. Spanish eggs were assumed to reflect an aquatic food chain due to their coastal location. Levels in eggs from terrestrial food chains examined in the same study (see Appendix 4**Error! Reference ource not found.**) were slightly higher in the Canadian sample but lower in the Spanish sample	Guerra *et al*. (2011)

Species	Tissue	Sample location and date	Limit of detection (LoD), µg/kg ww	Measured concentration	Comment	Reference
Glaucous Gull (*Larus hyperboreus*)	Liver	East Greenland (n = 4) (sampling dates not stated)	Not stated	Anti-DP: Arithmetic mean: 0.11 µg/kg ww Range: 0.07 – 0.18 µg/kg ww Syn-DP: Arithmetic mean: 0.02 µg/kg ww Range: 0.01 – 0.04 µg/kg ww	Samples originated from the specimen bank at Aarhus University, Denmark. Mean lipid content: 5.28 % (range: 3.32 – 6.42 %) Both anti- and syn-isomers were detected in all samples.	Vorkamp *et al*. (2015)
Ring-billed Gull (*Larus delawarensis*)	Liver & blood plasma	Deslauriers Island, near Montreal, Canada; April-June 2010	Anti-DP: 0.01 Syn-DP: 0.03	Liver samples (n=28) Total isomers: Mean 8.44 µg/kg ww [230 µg/kg lw] Anti-DP: Detected in all samples, mean 6.06 (range: 0.70−38.4) µg/kg ww Syn-DP: Detected in 93 % of samples, mean 2.38 (range: not detected−15.2) µg/kg ww Neither isomer detected in blood plasma (n=30)	Analysis by GC-MS. Highly industrialized area. No stereoselective enrichment detected.	Gentes *et al*. (2012)
Herring Gull (*Larus argentatus*)	Eggs	Six colonies in the Great Lakes region, North America; late April to early May 2004 (10-13 eggs per site, subsequently pooled)	Unclear (~ 0.01?)	Total isomers: 1.5 – 4.5 µg/kg ww	Analysis by GC-MS. Detected at every colony. Highest concentration was found in the Niagara River colony, close to the U.S. manufacturing site.	Gauthier *et a*l. (2007)

Species	Tissue	Sample location and date	Limit of detection (LoD), µg/kg ww	Measured concentration	Comment	Reference
Herring Gull (*Larus argentatus*)	Eggs	Seven colonies in the Great Lakes region, North America; late April to early May in 1982, 1987, 1992 & 1995-2006 (10-13 eggs per site, subsequently pooled)	Anti-DP: 0.01 (LoQ) Syn-DP: 0.02 (LoQ)	Total isomer concentrations were variable but below 15 µg/kg ww regardless of the sampling year or colony (with one exception: 18 µg/kg ww at one site in 1982). Anti-DP: 0.13-4.40 µg/kg ww Syn-DP: 0.31-1.40 µg/kg ww	Analysis by GC-MS. Highest concentration was detected in the Niagara River colony, close to the manufacturing site. Concentrations were generally higher after the mid-1990s for all sites. No temporal or spatial enrichment of either isomer relative to the commercial mixture	Gauthier & Letcher (2009)
Herring Gull (*Larus argentatus*)	Eggs	Twenty colonies across the Laurentian Great Lakes basin, Canada and United States. A total of 100 individual eggs collected in 2012 and 2013 and 15 egg pools collected in 2012.	Not given	Total DP: <LoD-54.6 µg/kg ww. The f_{anti} was in the range 0.6-0.72.	Syn- and anti-DP detectable in 83 % and 93 % respectively of the samples. The levels of total DP in the pooled eggs were significantly higher (p<0.05) than measured in pooled samples from the same colonies in 2006 and 2008 (220% higher in 2012 compared with 2006).	Su *et al.* (2015)

Species	Tissue	Sample location and date	Limit of detection (LoD), µg/kg ww	Measured concentration	Comment	Reference
Black Guillemot (*Cepphus grylle*)	Eggs	East Greenland (n = 4) (sampling dates not stated)	Not stated	Anti-DP: Arithmetic mean: 0.07 µg/kg ww Range: 0.01 – 0.19 µg/kg ww Syn-DP: Arithmetic mean: 0.01 µg/kg ww Range: 0.01 – 0.03 µg/kg ww	Samples originated from the specimen bank at Aarhus University, Denmark. Mean lipid content: 10.2 % (range: 9.66 – 10.7 %) Both anti- and syn-isomers were detected in all samples.	Vorkamp *et al*. (2015)
Black Guillemot (*Cepphus grylle*)	Eggs	Islands of Skúvoy (n=9) and Koltur (n=10), Faroe Islands (sampling date not stated).	Not stated	One pooled sample from each site. Total isomers: 0.012-0.033 µg/kg ww Anti-DP: 0.012-0.024 µg/kg ww Syn-DP: <0.008-0.0093 µg/kg ww	Analysis by GC-MS. Sites are remote from human activity.	Schlabach *et al*. (2011)
Common Guillemot (*Uria aalge*)	Eggs	Stora Karlsö, Sweden, 2009	Not stated	5 eggs, 2 sub-samples Total isomers: 0.079-0.083 µg/kg ww Anti-DP: 0.055-0.057 µg/kg ww Syn-DP: 0.023-0.026 µg/kg ww	Analysis by GC-MS. Site is remote from human activity.	Schlabach *et al*. (2011)

Species	Tissue	Sample location and date	Limit of detection (LoD), µg/kg ww	Measured concentration	Comment	Reference
Brown Skua (*Catharacta antarctica*) Gentoo Penguin (*Pygoscelis papua*) Adélie Penguin (*Pygoscelis adeliae*)	Carcass	King George Island, Antarctica, 2008 (skua) & 2009 (penguins)	Not stated	Both isomers detected in all samples (n=6). Mean concentrations of total isomers: Brown Skua (n=3): 4.55 µg/kg lw Penguins (n=3): 0.28 µg/kg lw 11.1 µg/kg lw in one skua sample	Analysis by GC-MS. Site is remote from human activity, though skuas may feed on research station refuse.	Kang *et al*. (2013) [ABST]
Seabirds including Common Eider (*Somateria mollisima*), European Shag (*Phalacrocorax aristotelis*) and European Herring Gull (*Larus argentatus*)	Eggs	Islands of Røst and Sklinna, Norway (n=18), May 2012-June 2012.	Not clear	Anti-DP: <LoD-0.08 µg/kg Syn-DP: <LoD	Anti-DP detected in 2 samples. Syn-DP not detectable	Huber *et al.* (2015a&b) .

Species	Tissue	Sample location and date	Limit of detection (LoD), µg/kg ww	Measured concentration	Comment	Reference
Common Kingfisher (*Alcedo atthis*)	Pectoral muscle	E-waste recycling site & reference site (Dinghu Mountain) in the Pearl River Delta, Guangdong Province, southern China; March-July 2010.	Anti-DP: 0.59 (lw) Syn-DP: 0.14 (lw)	E-waste recycling site (n = 22) Total isomers: median 58 (range: 29–150) µg/kg lw Reference site (n = 17) Total isomers: median 3.9 (range: 0.98–13) µg/kg lw Anti-DP-1Cl was detected in all samples collected from the e-waste recycling site (range: 0.22-13 µg/kg lw), but only in 17 % of the birds from the reference site (range: <0.09-0.36 µg/kg lw). Anti-DP-2Cl was not detected in any sample (LoD 0.01 µg/kg lw).	Analysis by GC-MS. The e-waste site is in a heavily industrialized area. The reference site is in a relatively non-contaminated agricultural area. The anti- isomer fractions from the e-waste recycling site were significantly smaller than those from the reference site, and were negatively correlated to logarithm of total isomer concentrations (r^2 = 0.41, $p < 0.0001$).	Mo *et al*. (2013)

Species	Tissue	Sample location and date	Limit of detection (LoD), µg/kg ww	Measured concentration	Comment	Reference
MAMMALS						
Eurasian Otter (*Lutra lutra*)	Liver	Adult and subadult male and female animals found dead in England and Wales during 2010 (n=30) & 2011 (n=34).	Anti-DP: 0.338 (2010) and 0.428 (2011) Syn-DP: 0.338 (2010) and 0.508 (2011)	Detected in 3 out of 64 samples Anti-DP: 0.395, 0.449 & 0.967 µg/kg ww. Syn-DP: not detected in any sample (n=64)	Analysed by GC-MS. Lipid concentration not given. The animals with positive detects were from widely separated areas.	Walker *et al*. (2013) & CEH (2014)
Franciscana (dolphin) (*Pontoporia blainvillei*)	Liver	Stranded or bycaught animals around south-eastern and southern coast of Brazil between 1994 and 2008.	Not stated	Detected in 15 out of 20 animals. Total isomers: mean 1.53 (range: not detected - 6.26) µg/kg lw No correlations were found between substance concentration and lipid content. Anti-DP-1Cl and anti-DP-2Cl were not detected in any sample (LoD not stated).	Analysed by GC-MS.	de la Torre *et al*. (2012)
Harbour Porpoise (*Phocoena phocoena*)	Blubber	Stranded or bycaught animals around UK coasts during 2008.	Anti-DP: 0.12-0.14 Syn-DP: 0.12-0.15	Anti-DP detected up to 0.36 µg/kg ww in 18 out of 21 animals. Syn-DP detected up to 0.17 µg/kg ww in 7 out of 21 animals.	Analysed by GC-MS/MS.	Law *et al*. (2013)

Species	Tissue	Sample location and date	Limit of detection (LoD), µg/kg ww	Measured concentration	Comment	Reference
Short-beaked Common Dolphin (*Delphinus delphis*)	Blubber	Samples collected from live animals by biopsy from the Gulf of Cádiz, Spain (n=8) during February 2012	Not stated	Total isomers: <LoD-27.1 µg/kg lw Anti-DP: <LoD-12.9 µg/kg lw Syn-DP: <LoD-14.2 µg/kg lw	Analytical method not stated. Based on a comparison of $\delta^{13}C$ and $\delta^{15}N$ ratios, the animals were at a lower trophic position and feeding on a different food source than the samples of *Tursiops truncatus* collected at the same time.	Eljarrat (2014) [ABST]
Short-beaked Common Dolphin (*Delphinus delphis*)	Blubber	Samples collected from live animals by biopsy from the Gulf of Cádiz (n=15) and Strait of Gibraltar, Spain (n=2) during 2012	0.01 µg/kg lw for anti-DP 0.02 µg/kg lw for syn-DP	Gulf Anti-DP: mean 1.83 µg/kg lw (range<LoD-12.9 µg/kg lw) Syn-DP: mean 2.74 µg/kg lw (range <LoD-14.2 µg/kg lw) f_{anti}; mean 0.36 (range 0.11-0.54) Strait Anti-DP: mean 5.94 µg/kg lw (range 0.37-11.6 µg/kg lw) Syn-DP: mean 7.60 µg/kg lw (range 0.31-14.9 µg/kg lw) f_{anti}; mean 0.47 (range 0.44-0.49)	Analysed by GC-MS/MS. Based on a comparison of $\delta^{15}N$ ratios, the *Tursiops truncatus* were at a higher trophic position than the samples of *Globicephala melas* and *Delphinus delphis* collected at the same time.	Barón *et al.* (2015a) [May be same study as Eljarrat (2014) above]

Species	Tissue	Sample location and date	Limit of detection (LoD), µg/kg ww	Measured concentration	Comment	Reference
Bottlenose Dolphin (*Tursiops truncatus*)	Blubber	Samples collected from live animals by biopsy from the Gulf of Cádiz and Strait of Gibraltar, Spain (n=20) during February 2012	Not stated	Gulf Total isomers: <LoD-21.1 µg/kg lw Anti-DP: <LoD-9.68 µg/kg lw Syn-DP: <LoD-11.4 µg/kg lw Strait Total isomers: <LoD-5.0 µg/kg lw Anti-DP: <LoD-5.0 µg/kg lw Syn-DP: <LoD-5.44 µg/kg lw	Analytical method not stated. Based on a comparison of $\delta^{13}C$ and $\delta^{15}N$ ratios, the animals were at a higher trophic position and feeding on a different food source than the samples of *Delphinus delphis* collected at the same time. Animals from the Gulf of Cádiz occupied a slightly higher trophic position from those from the Strait of Gibraltar.	Eljarrat (2014) [ABST]
Bottlenose Dolphin (*Tursiops truncatus*)	Blubber	Samples collected from live animals by biopsy from the Gulf of Cádiz (n=20) and Strait of Gibraltar, Spain (n=20) during 2012	0.01 µg/kg lw for anti-DP 0.02 µg/kg lw for syn-DP	Gulf Anti-DP: mean 2.15 µg/kg lw (range<LoD-10.1 µg/kg lw) Syn-DP: mean 2.79 µg/kg lw (range <LoD-12.6 µg/kg lw) f_{anti}; mean 0.49 (range 0.16-0.79) Strait Anti-DP: mean 2.10 µg/kg lw (range<LoD-10.6 µg/kg lw) Syn-DP: mean 2.48 µg/kg lw (range <LoD-8.1 µg/kg lw) f_{anti}; mean 0.48 (range 0.02-0.87)	Analysed by GC-MS/MS. Based on a comparison of $\delta^{15}N$ ratios, the samples of *Tursiops truncatus* were at a higher trophic position than the samples of *Globicephala melas* and *Delphinus delphis* collected at the same time.	Barón *et al.* (2015a) [May be same study as Eljarrat (2014) above]

Species	Tissue	Sample location and date	Limit of detection (LoD), µg/kg ww	Measured concentration	Comment	Reference
Long-finned Pilot Whale (*Globicephala melas*)	Blubber	Samples collected from live animals by biopsy from the Strait of Gibraltar, Spain (n=10) during 2012	0.01 µg/kg lw for anti-DP 0.02 µg/kg lw for syn-DP	Anti-DP: mean 1.18 µg/kg lw (range<LoD-2.93 µg/kg lw) Syn-DP: mean 1.50 µg/kg lw (range <LoD-4.23 µg/kg lw) f_{anti}; mean 0.49 (range 0.33-0.71)	Analysed by GC-MS/MS. Based on a comparison of $\delta^{15}N$ ratios, the samples of *Tursiops truncatus* were at a higher trophic position than the samples of *Globicephala melas* and *Delphinus delphis* collected at the same time.	Barón *et al.* (2015a)
Common Bottlenose Dolphin (*Tursiops truncatus*)	Blubber and brain	Samples collected from stranded individuals from the Alboran Sea between 2004 and 2011 (n=1)	0.01 µg/kg lw for anti-DP 0.02 µg/kg lw for syn-DP	Blubber Anti-DP: <LoD? Syn-DP: <LoD? Brain Anti-DP: <LoD? Syn-DP: <LoD?	Analysed by GC-MS/MS.	Barón *et al.* (2015b)
Common Dolphin (*Delphinus delphis*)	Blubber and brain	Samples collected from stranded individuals from the Alboran Sea between 2004 and 2011 (n=10)	0.01 µg/kg lw for anti-DP 0.02 µg/kg lw for syn-DP	Blubber Anti-DP: <LoD-3.82 µg/kg lw Syn-DP: <LoD-3.68 µg/kg lw Brain Anti-DP: <LoD-1.44 µg/kg lw Syn-DP: <LoD-1.55 µg/kg lw	Analysed by GC-MS/MS. Detected in 2 samples	Barón *et al.* (2015b)
Risso's Dolphin (*Grampus griseus*)	Blubber and brain	Samples collected from stranded individuals from the Alboran Sea between 2004 and 2011 (n=1)	0.01 µg/kg lw for anti-DP 0.02 µg/kg lw for syn-DP	Blubber Anti-DP: <LoD Syn-DP: <LoD Brain Anti-DP: 0.37? µg/kg lw Syn-DP: 0.36? µg/kg lw	Analysed by GC-MS/MS.	Barón *et al.* (2015b)

Species	Tissue	Sample location and date	Limit of detection (LoD), µg/kg ww	Measured concentration	Comment	Reference
Striped Dolphin (*Stenella coeruleoalba*)	Blubber and brain	Samples collected from stranded individuals from the Alboran Sea between 2004 and 2011 (n=11)	0.01 µg/kg lw for anti-DP 0.02 µg/kg lw for syn-DP	Blubber Anti-DP: <LoD-10.4 Syn-DP: <LoD-10.0 Brain Anti-DP: <LoD-2.33 µg/kg lw Syn-DP: <LoD-2.62 µg/kg lw	Analysed by GC-MS/MS. Detected in 6 blubber and 2 brain samples.	Barón *et al.* (2015b)
Long-fined Pilot Whale (*Globicephala melas*)	Blubber and brain	Samples collected from stranded individuals from the Alboran Sea between 2004 and 2011 (n=3)	0.01 µg/kg lw for anti-DP 0.02 µg/kg lw for syn-DP	Blubber Anti-DP: <LoD-0.33 µg/kg lw Syn-DP: <LoD-0.31 µg/kg lw Brain Anti-DP: <LoD Syn-DP: <LoD	Analysed by GC-MS/MS. Detected in 2 samples of blubber but not detected in brain.	Barón *et al.* (2015b)
Beluga Whale (*Delphinapterus leucas*)	Blubber	Samples collected in 2000 and 2010 at Hendrickson Island, Canadian Arctic (n=8)	5-15 ng/kg lw	Not detected	Analysed by GC-HRMS.	Shen *et al*. (2012).
Indo-Pacific Humpback Dolphin (*Sousa chinensis*)	Blubber	Samples collected from stranded animals in Hong Kong, South China Sea between 2003 and 2012 (n=23)	-	Detected in all samples Total isomers: 1.74-63.7 µg/kg lw f_{anti}: 0.6	No significant difference between genders was found. This species inhabits waters adjacent to the mouth of the Pearl River estuary, and eats mainly fish.	Zhu *et al*. (2014)

Species	Tissue	Sample location and date	Limit of detection (LoD), µg/kg ww	Measured concentration	Comment	Reference
Finless Porpoise (*Neophocaena phocaenoides*)	Blubber	Samples collected from stranded animals in Hong Kong, South China Sea between 2003 and 2012 (n=38)	-	Detected in all samples Total isomers: 0.45-5.06 µg/kg lw f_{anti}: 0.5	No significant difference between genders was found. This species inhabits waters away from river estuaries, and has a high proportion of celaphopods in its diet.	Zhu *et al*. (2014)

Species	Tissue	Sample location and date	Limit of detection (LoD), µg/kg ww	Measured concentration	Comment	Reference
Ringed Seal (*Phoca hispida*)	Blubber	East (n = 5) and West (n = 4) Greenland (sampling dates not stated)	Not stated (0.01 assumed)	East Greenland samples: Anti-DP: Arithmetic mean: 0.42 µg/kg ww Range: 0.04 – 1.84 µg/kg ww Syn-DP: Arithmetic mean: 0.10 µg/kg ww Range: < 0.01 – 0.42 µg/kg ww West Greenland samples: Anti-DP: Arithmetic mean: 0.07 µg/kg ww Range: 0.05 – 0.08 µg/kg ww Syn-DP: Arithmetic mean: 0.02 µg/kg ww Range: 0.01 – 0.03 µg/kg ww	Samples originated from the specimen bank at Aarhus University, Denmark. Mean lipid content: 93.1 % (range: 92.2 – 93.9 %) [East Greenland], 94.1 % (range: 91.9 – 96.2 %) [West Greenland] Both anti- and syn-isomers were detected in eight out of nine samples.	Vorkamp *et al*. (2015)
Polar Bear (*Ursus maritimus*)	Adipose	East (n = 5) and West Greenland (sampling dates not stated)	Not stated	Anti-DP: Arithmetic mean: 0.06 µg/kg ww Range: 0.03 – 0.08 µg/kg ww Syn-DP: Arithmetic mean: 0.02 µg/kg ww Range: 0.01 – 0.03 µg/kg ww	Samples originated from the specimen bank at Aarhus University, Denmark. Mean lipid content: 86.4 % (range: 77.2 – 93.8 %) Both anti- and syn-isomers were detected in all samples.	Vorkamp *et al*. (2015)

APPENDIX 4 MEASURED CONCENTRATIONS OF DECHLORANE PLUS IN BIOTA ASSOCIATED WITH THE TERRESTRIAL ENVIRONMENT

Species	Tissue	Sample location and date	Limit of detection (LoD), µg/kg wet wt.	Measured concentration	Comment	Reference
Light-vented Bulbul (*Pycnonotus sinensis*), n=16 Yellow-bellied Prinia (*Prinia flaviventris*), n=9 Plain Prinia (*Prinia inornata*), n=4 Dark Green White-eye (*Zosterops japonicus*), n=4	Eggs	Samples were collected between 2010 and 2012 from an ecological station in the Pearl River Delta, southern China (n=33)	3.26 µg/kg lw	Total isomers (median, range): Bulbul: 15 (4.6–30) µg/kg lw Yellow-bellied Prinia: 34 (24–58) µg/kg lw Plain Prinia: 141 (69–268) µg/kg lw White-eye: 33 (14–87) µg/kg lw Anti-DP-1Cl: <0.028 – 0.86 µg/kg lw (detected in 73 % of eggs) Anti-DP-2Cl: <0.035 µg/kg lw	No direct emission source exists at the sampling site. The bulbul eats plant matter, and had significantly lower levels of flame retardants than the insectivores sampled in the same study. There was a significant negative relationship between the fraction of anti-DP and DP concentrations, suggesting that DP levels play an important role in determining the isomeric composition.	Sun *et al*. (2014)

Species	Tissue	Sample location and date	Limit of detection (LoD), µg/kg wet wt.	Measured concentration	Comment	Reference
White Stork (*Ciconia ciconia*)	Eggs (failed)	One colony near Madrid sampled in the 2005 breeding season (10 eggs, urban/ industrial area) and another in the Doñana National Park sampled in the 1999–2000 breeding seasons (23 eggs, rural area), Spain.	Anti-DP: 0.0008 (limit of quantitation, LoQ) Syn- DP: 0.0005 (LoQ)	Dechlorane Plus was found in all eggs (n = 33). *Urban colony* Total isomers: average 0.44 (range: 0.06–1.40) µg/kg ww Anti- isomer: average 0.27 (range: <LoQ–1.0) µg/kg ww Syn- isomer: average 0.134 (range: <LoQ–0.38) µg/kg ww *Rural colony* Total isomers: average 0.10 (range: 0.002–0.47) µg/kg ww Anti- isomer: average 0.07 (range: 0.0006–0.34) µg/kg ww Syn- isomer: average 0.05 (range: 0.002–0.14) µg/kg ww	Analysed by GC-MS. Measured levels in procedural blanks were subtracted from the corresponding batch of samples associated with each blank. Care was taken to minimize exposure to UV light. Mean lipid content: 7.18 ±0.73 % (Madrid) and 7.32±0.96 % (Doñana). A possible transformation product (anti-[DP-1Cl]) was found in about 10% of the samples from each colony. 1,5-DPMA and anti-[DP-2Cl] were not detected above their LoQs in any sample (LoQ not provided).	Muñoz-Arnanz *et al*. (2010)
White Stork (*Ciconia ciconia*)	Eggs	Spain	-	0.31 µg/kg ww in Madrid 0.08 µg/kg ww in a national park	This is presumably an early report of the study mentioned above.	Muñoz-Arnanz *et al.* (2010) [ABST]

Species	Tissue	Sample location and date	Limit of detection (LoD), µg/kg wet wt.	Measured concentration	Comment	Reference
Peregrine Falcon (*Falco peregrinus*)	Eggs	Great Lakes Basin, Canada (Lake Superior, Lake Ontario and the St. Lawrence River); between 2007 and 2009 (n=10) Guadalajara, Spain; from 2003 to 2006 (n=5)	0.012 µg/kg lw	Dechlorane Plus was detected in all of the samples (n=15). Geometric means with ranges are: Canada 38.4 (7.5 – 209) µg/kg lw Spain 0.5 (0.03 – 3.6) µg/kg lw Anti-DP-1Cl and anti-DP-2Cl were detected in almost all of the eggs from Canada (n=10 and 9, respectively), with concentrations in the range 1.1 to 2.3 µg/kg lw. However, in the Spanish samples, anti-DP-1Cl was below the limit of quantification in all five eggs, and anti-DP-2Cl was not detected. 1,5-DPMA was detected in all eggs with concentrations in the range 1.2 – 1 660 µg/kg lw (Canada) and 1.7 – 37 µg/kg lw (Spain).	Analysis was by GC-HRMS. The Canadian results are thought to reflect local exposure based on proximity to one of the known production sites. Canadian food chains were determined by prey analysis. The relative concentration of the anti-isomer in the eggs of birds with a known terrestrial diet was significantly higher in the Spanish samples compared to their Canadian counterparts. Levels in eggs from aquatic food chains examined in the same study (see Appendix 3) were slightly lower in the Canadian sample but higher in the Spanish sample.	Guerra *et al*. (2011)

Species	Tissue	Sample location and date	Limit of detection (LoD), µg/kg wet wt.	Measured concentration	Comment	Reference
xxi) American Kestrel (*Falco sparverius*)	Egg	xxii) Samples collected during 2008 from Grimsby, Niagara-on-the-Lake, St. George and Tillsonburg, Ontario, Canada (10 eggs in 3 pools for each location)	Exact value not stated (in the range 0.01-0.78)	Anti-DP: 0.3-2.4 µg/kg ww at Niagara-on-the-Lake, <LoQ-0.1 µg/kg ww at St. George, not detected at Grimsby or Tillsonburg. Syn-DP: <LoD at all sites except Niagara-on-the-Lake (where maximum concentration was 0.8 µg/kg ww).	Niagara-on-the-Lake is approximately 15 km from the only North American manufacturing site.	xxiii) Chen *et al*. (2012a)
Eurasian Sparrowhawk (*Accipiter nisus*)	Pectoral muscle and liver	Beijing, China; March 2004 to January 2006	Anti-DP: 0.07-0.7 µg/kg lw Syn-DP: 0.1-1 µg/kg lw	Both isomers detected in all individuals (n=11). Muscle Total isomers: median 105 (range: 26–1 320) µg/kg lw Liver Total isomers: median 203 (range: 16–2 400) µg/kg lw DP-1Cl concentrations were <1.2–83 µg/kg lw in muscle and <1–78 µg/kg lw in liver. DP-2Cl was not detectable in any tissue (LoD not stated).	Analysis was by GC-MS. Significant positive correlations between the anti- isomer:total isomer concentration ratio and the total isomer concentration suggest that burdens could be substantially driven by the accumulation of the anti- isomer.	Chen *et al*. (2013b)

Species	Tissue	Sample location and date	Limit of detection (LoD), µg/kg wet wt.	Measured concentration	Comment	Reference
Japanese Sparrowhawk (*Accipiter gularis*)	Pectoral muscle and liver	Beijing, China; March 2004 to January 2006	Anti-DP: 0.07-0.7 µg/kg lw Syn-DP: 0.1-1 µg/kg lw	Both isomers detected in all individuals (n=6). Muscle Total isomers: median 85 (range: 40–990) µg/kg lw Liver Total isomers: median 160 (range: 40–430) µg/kg lw DP-1Cl concentrations were 3–103 µg/kg lw in muscle and <1.2–770 µg/kg lw in liver. DP-2Cl was not detectable in any tissue (LoD not stated).	Analysis was by GC-MS.	Chen *et al*. (2013b)

Species	Tissue	Sample location and date	Limit of detection (LoD), µg/kg wet wt.	Measured concentration	Comment	Reference
Common Kestrel (*Falco tinnunculus*)	Pectoral muscle and liver	Beijing, China; March 2004 to January 2006	Anti-DP: 0.07-0.7 µg/kg lw Syn-DP: 0.1-1 µg/kg lw	Both isomers detected in all individuals (n=6). Muscle Total isomers: median 810 (range: 30 – 2 130) µg/kg lw Liver Total isomers: median 550 (range: 25 – 3 820) µg/kg lw DP-1Cl concentrations were 1.2–210 µg/kg lw in muscle and 1–160 µg/kg lw in liver. DP-2Cl was not detectable in any tissue (LoD not stated).	Analysis was by GC-MS.	Chen *et al*. (2013b)

Species	Tissue	Sample location and date	Limit of detection (LoD), µg/kg wet wt.	Measured concentration	Comment	Reference
(Oriental) Scops Owl (*Otus sunia*, n=6)	Pectoral muscle and liver	Beijing, China; March 2004 to January 2006	Anti-DP: 0.07-0.7 µg/kg lw Syn-DP: 0.1-1 µg/kg lw	Anti- isomer detected in all individuals (n=6). Muscle Total isomers: median 40 (range: 5–150) µg/kg lw Liver Total isomers: median 71 (range: 15–140) µg/kg lw DP-1Cl levels were not reported due to the number of results below the LoD. DP-2Cl was not detectable in any tissue (LoD not stated).	Analysis was by GC-MS.	Chen *et al*. (2013b)
Long-eared Owl (*Asio otus*, n=6)	Pectoral muscle and liver	Beijing, China; March 2004 to January 2006	Anti-DP: 0.1 µg/kg lw (muscle) 2 µg/kg lw (liver) Syn–DP: 0.2 µg/kg lw 2 µg/kg lw (liver)	Both isomers not detected in all individuals (n=6). Muscle Total isomers: median 0.71 (range: 0.62–0.84) µg/kg lw Liver Total isomers: median 0.60 (range: 0.60–0.67) µg/kg lw DP-1Cl concentrations were <0.1–20 µg/kg lw in muscle and <0.2–120 µg/kg lw in liver. DP-2Cl	Analysis was by GC-MS.	Chen *et al*. (2013b)

Species	Tissue	Sample location and date	Limit of detection (LoD), µg/kg wet wt.	Measured concentration	Comment	Reference
				was not detectable in any tissue (LoD not stated).		
Little Owl (*Athene noctua*)	Pectoral muscle and liver	Beijing, China; March 2004 to January 2006	Anti-DP: 0.07-0.7 µg/kg lw Syn-DP: 0.1-1 µg/kg lw	Both isomers detected in all individuals (n=6). Muscle Total isomers: median 240 (range: 50–1 500) µg/kg lw Liver Total isomers: median 480 (range: 23–1 740) µg/kg lw DP-1Cl concentrations were <0.9–85 µg/kg lw in muscle and <1.3–85 µg/kg lw in liver. DP-2Cl was not detectable in any tissue (LoD not stated).	Analysis was by GC-MS.	Chen *et al*. (2013b)
Domestic Chicken (*Gallus gallus domesticus*)	Eggs	Three recycling sites and a reference site in an e-waste recycling region of southern China; July 2010	LoQ: in the range 0.08 to 6.26 µg/kg lw	Detected in >50% of samples (n=41) Total isomers: site means were in the range 124–3 290 µg/kg lw	Birds were free-range. No stereoisomeric selectivity was observed.	Zheng *et al*. (2012)

Species	Tissue	Sample location and date	Limit of detection (LoD), µg/kg wet wt.	Measured concentration	Comment	Reference
European Starling (*Sturnus vulgaris*)	Egg	Samples collected during 2008 from Grimsby, Niagara-on-the-Lake, St. George and Tillsonburg, Ontario, Canada (10 eggs in 3 pools for each location)	Exact value not stated (in the range 0.01-0.78)	Anti-DP: <0.2-6.0 µg/kg ww at Niagara-on-the-Lake, <LoQ-0.2 µg/kg ww at Grimsby, not detected at St. George or Tillsonburg. Syn-DP: <LoD at all sites except Niagara-on-the-Lake (where maximum concentration was 4.0 µg/kg ww).	Niagara-on-the-Lake is approximately 15 km from the only North American manufacturing site.	xxiv) Chen *et al*. (2012a)
European Starling (*Sturnus vulgaris*)	Egg	Samples collected during 2009, 2010 and 2011 in the vicinity of five Canadian cities: Vancouver, British Columbia (3 sites); Calgary, Alberta (5 sites); Hamilton, Ontario (6 sites); Montréal, Québec (4 sites); and Halifax, Nova Scotia (3 sites), covering several land use types (districts of industrial activity within city limits, landfill sites and rural agricultural sites, as well as a non-urban national reference site).	Not stated	Detectable in 41 % of the examined egg pool homogenates (representing up to 10 eggs from each site and year), with concentrations (total isomers) up to 24 µg/kg ww. At the 16 sites for which median concentrations could be derived, the median was in the range 0.1 – 2.2 µg/kg ww. The mean f_{anti} was 0.80 (range: 0.30 – 0.91).	Analysis was by GC-MS. Based on an analysis of PBDEs in the same samples, landfills were found to be an important source of exposure.	Chen *et al*. (2013a)

Species	Tissue	Sample location and date	Limit of detection (LoD), µg/kg wet wt.	Measured concentration	Comment	Reference
Domestic Dog (*Canis familiaris*)	Blood serum	Samples collected at a veterinary clinic in Bloomington, Indiana, USA (n = 18)	-	Detected in 14 samples (78 %). Total isomers Average: 0.032±0.006 µg/kg ww (maximum: 0.133 µg/kg ww)	All of the dogs lived mainly indoors. There was no significant correlation between the levels in serum and dog food (average concentration in food: 0.030±0.006 µg/kg ww, n = 16). The paper notes that the average concentration in dry cat food was 0.086±0.014 µg/kg ww (unpublished data).	Venier & Hites (2011)
Lichens	Whole lichen	Southeast Tibetan Plateau, July 2013. A total of 26 samples collected across 3 mountain transects.	Limit of quantification was 0.030 µg/kg dw	Total isomers: mean 0.318 µg/kg dw (range <LoD-1.1 µg/kg dw) The mean f_{anti} was 0.71 (range 0.46-0.83).	Analysis was by GC-MS-NCI. Detectable in 89 % of the samples. The concentrations showed a decreasing trend with increasing altitude.	Yang *et al*. (2016)
Moss (*Hytocomium splendens*)	Whole sample	Incineration facilities at Tórshavn and Leirvíc, Faroe Islands, 2009 (1 sample from each site).	Not stated but probably	Anti-DP: 0.04-0.12 µg/kg dw Syn-DP: 0.02-0.05 µg/kg dw	Analysis as by GC-MS.	Schlabach *et al*. (2011)

Species	Tissue	Sample location and date	Limit of detection (LoD), µg/kg wet wt.	Measured concentration	Comment	Reference
Moss, dung (reindeer and bird)	Whole sample	Ny-Ålesund, Svalbard (78°55'N, 11°56'E).	0.004-0.054 (dung) 0.00057-0.076 (soil)	Moss: Anti-DP: mean 0.0004 µg/kg dw (range <LoD-0.0009 µg/kg) Syn-DP: mean 0.001 µg/kg dw (range 0.0001-0.0025 µg/kg dw) f_{anti} = 0.27 Dung: Anti-DP: mean 0.171 µg/kg dw (range 0.0017-0.524 µg/kg) Syn-DP: mean 0.087 µg/kg dw (range 0.0035-0.369 µg/kg dw) The f_{anti} was 0.66 in reindeer dung and 0.67 in bird dung.	Analysis was by GC-MS-NCI. Samples of moss, dung (and surface soil) were collected simultaneously. Sediment and seawater samples were also taken at King's Bay. Na *et al.* (2015) considered the low f_{anti} values found in moss (and also water, sediment, soil) may reflect degradation of anti-DP during long-range transport, possibly by UV. The f_{anti} value in the dung samples was close to that in commercial products and suggests that the migration behaviour of the organisms may be the main source of transport for these organisms.	Na *et al*. (2015)

Species	Tissue	Sample location and date	Limit of detection (LoD), µg/kg wet wt.	Measured concentration	Comment	Reference
Green Onion (*Allium fistulosum*) (n = 12) Lettuce (*Lactuca sativa*) (n = 8) Garlic Chives (*Allium tuberosum*) (n = 18) Pak Choi Cabbage (*Brassica rapa chinensis*) (n = 15) Field Mustard (*Brassica rapa*) (n = 10) Common Wheat (*Triticum aestivum*) (n = 50) Maize (*Zea mays*) (n = 40) Soybean (*Glycine max*) (n = 40)	Leaves (vege-tables) or grain (wheat, maize, soy-bean)	Huai'an, Jiangsu province, China; May 2010.	Anti-DP: 0.135 Syn-DP: 0.120	Total isomers: Mean for vegetables: 1 038 (range of means for each species: 305 to 2 720) µg/kg ww Mean for grains: 877 (range of means for each species: 498 to 1 370) µg/kg ww	Analysis by GC- high resolution MS. The sampling site was in close proximity to the Chinese manufacturing facility.	Wang *et al*. (2013)

Species	Tissue	Sample location and date	Limit of detection (LoD), µg/kg wet wt.	Measured concentration	Comment	Reference
Rat (species not specified)	Liver	Oslo / Akershus area of Norway (caught by a pest control company) (date not provided) (n = 5)	Anti-DP: 0.14 Syn-DP: 0.03	Anti-DP: <0.14 – 0.27 µg/kg ww Syn-DP: 0.03 – 0.13 µg/kg ww		Schlabach *et al*. (2017)

APPENDIX 5 OTHER EUROPEAN MONITORING STUDIES

This appendix provides a brief summary of data on the environmental occurrence of Dechlorane Plus that is not directly relevant to this report. The information has been obtained from abstracts, and a detailed review has not been performed. The focus is on data obtained in Europe; studies from outside Europe are listed in the Annex.

Compartment	Location	Findings	Reference
Atmosphere	Stockholm, Sweden	Urban air sampled in November 2009 and January 2010: 1.4 – 1.5 pg/m^3 Deposition fluxes were estimated from the monthly average data as 0.50 – 0.55 ng/m^2/d	Kaj *et al*. (2010)
	Stockholm, Sweden	Urban air sampled in November 2009 and January 2010. November 2009: Anti-DP 0.13 pg/m^3 and syn-DP 0.039 pg/m^3. January 2010: Anti-DP 0.065 pg/m^3 and syn-DP 0.044 pg/m^3.	Schlabach *et al*. (2011)
	Oslo, Norway	Urban air sampled in 2009: Anti-DP 16-120 pg/m^3 and syn-DP 12-42 pg/m^3.	Schlabach *et al*. (2011)
	Råö, Sweden	Remote location on the Swedish west coast sampled in November 2009 and January 2010. November 2009: Anti-DP 0.43 pg/m^3 and syn-DP 0.15 pg/m^3. January 2010: Anti-DP 0.082 pg/m^3 and syn-DP 0.047 pg/m^3.	Schlabach *et al*. (2011)
	Lille Valby and Copenhagen, Denmark	Rural air at Lille Valby: Anti-DP 7.4 pg/m^3 and syn-DP 2.0 pg/m^3. Urban air at Copenhagen: Anti-DP 2.0 pg/m^3 and syn-DP 0.68 pg/m^3. The dates of sampling were not given.	Schlabach *et al*. (2011)
	Spain	Passive, integrated sampling 11 pg/m^3 (Madrid) 0.8 pg/m^3 (Rural)	de la Torre *et al*. (2010) [ABST]
Tree bark	Halle, Germany (May 2005) Trieste, Italy (April 2005)	Average ± standard deviation: 0.032 ± 0.019 µg/kg (Germany) 0.067 ± 0.032 µg/kg (Italy) Levels were about ten times lower than samples collected in the USA and Asia, presumably reflecting differences in emission sources.	Qiu *et al*. (2008) [ABST]; Qiu & Hites (2008)
	Birkeness, Norway; Košetice, Czech Republic; Malin Head, Ireland; Reykjavik, Iceland; 2009-2010 (n=3-4)	Average ± standard deviation: 9.12 ± 4.89 ng/g lw (Norway) 4.36 ± 1.98 µg/kg lw (Czech Republic) 4.81 ± 1.15 µg/kg lw (Ireland) 1.02 ± 0.25 µg/kg lw (Iceland)	Salamova & Hites (2013)

Compartment	Location	Findings	Reference
Sewage sludge	Sweden	4 urban wastewater treatment plants (WWTP) sampled once in 2009 Total isomers: 6.9–21 µg/kg dw (WWTP effluent concentrations ranged from <0.1 to 0.83 ng/L)	Kaj *et al*. (2010)
	Sweden	2 urban WWTP sampled once in 2009. Anti-DP: 3.5-5.6 µg/kg dw Syn-DP: 2.2-3.8 µg/kg dw 2 sludge samples from landfills sampled once in 2009-2010. Anti-DP: 5.1-21 µg/kg dw Syn-DP: 4.6-5.5 µg/kg dw May be the same samples as Kaj *et al.* (2010) above.	Schlabach *et al*. (2011)
	Finland	3 samples from urban wastewater treatment plants sampled once in 2009. Anti-DP: 5.0-7.4 µg/kg dw Syn-DP: 3.1-5.8 µg/kg dw 3 samples from storm water outflows of 2 landfill sites and 1 industrial area sampled once in 2009. Anti-DP: 0.05-11 µg/kg dw Syn-DP: <0.01-5.1 µg/kg dw	Schlabach *et al*. (2011)
	Norway	2 samples from an urban wastewater treatment plant sampled once in 2009. Anti-DP: 1.5-7.1 µg/kg dw Syn-DP: 0.85-1.3 µg/kg dw 2 samples from recycling facilities sampled once in 2009. Anti-DP: 0.21-1.4 µg/kg dw Syn-DP: 0.10-0.76 µg/kg dw	Schlabach *et al*. (2011)
		2 urban wastewater treatment plants sampled 5 times in 2016. Anti-DP: 3.0–3.6 µg/kg dw in sludge. Effluent concentrations <0.4-0.53 ng/L Syn-DP: 0.91–1.1 µg/kg dw in sludge. Effluent concentrations <0.1-0.16 ng/L	Schlabach *et al*. (2017)
	Denmark	2 samples from urban wastewater treatment plants sampled once in 2009. Anti-DP: 5.7-20 µg/kg dw Syn-DP: 3.5-14 µg/kg dw	Schlabach *et al*. (2011)
	Faroe Islands	2 samples from urban wastewater treatment plants sampled once in 2009. Anti-DP: 12-25 µg/kg dw	Schlabach *et al*. (2011)

Compartment	Location	Findings	Reference
		Syn-DP: 3.4-7.1 µg/kg dw	
	Iceland	2 samples from urban wastewater treatment plants sampled once in 2009. Anti-DP: 0.56-0.73 µg/kg dw Syn-DP: 0.24-0.25 µg/kg dw	Schlabach *et al*. (2011)
	Spain	31 WWTP sampled April to June 2006. Total isomers: 2.45–93.8 µg/kg dw	de la Torre *et al*. (2011)
		Ebro river basin: up to 18.8 µg/kg dw	Barón *et al*. (2012)
		7 WWTP sludges from the river basins of the Rivers Ebro and Llobregat in 2010: 2.58 – 18.8 µg/kg dw	Barón *et al*. (2014b)
	Germany	12 municipal waste water treatment plants from Bavaria from 2009. Total isomers: 6.9-72.8 µg/kg dw (mean 22.0 µg/kg dw)	Körner *et al.* (2011) [ABST]
Sediment	Spain	Ebro river basin: up to 1.61 µg/kg dw	Barón *et al*. (2012)
		33 sediments from the river basins of the Rivers Ebro and Llobregat in 2010: not quantified – 2.2 µg/kg dw (arithmetic mean: 0.44 µg/kg dw)	Barón *et al*. (2014b)
	Sweden	Sediments downstream of a WWTP in Stockholm. Sampled near the effluent source, 1 km downstream and 20 km downstream in 2009. Anti-DP: 0.16-2.0 µg/kg dw Syn-DP: 0.08-0.99 µg/kg dw	Schlabach *et al*. (2011)
	Finland	Marine sediment from two coastal estuary bays at Helsinki and Pori, and a sample from Lake Pyhäjärvi downstream of the city of Tampere, each sampled once in 2009. Anti-DP: 0.21-0.66 µg/kg dw Syn-DP: 0.06-0.22 µg/kg dw	Schlabach *et al*. (2011)
	Norway	Sediment for Åsefjorden sampled once in 2009. Anti-DP: 0.92 µg/kg dw Syn-DP: 0.28 µg/kg dw	Schlabach *et al*. (2011)
		Sediment in Lake Mjøsa sampled in 5 locations once in 2016. Neither isomer detected (<6 and <1 µg/kg dw for anti-DP and syn-DP)	Schlabach *et al*. (2017)
	Denmark	Marine sediment from Roskilde Marina and Fornaes, each sampled once in 2009. Anti-DP: 0.0049-1.6 µg/kg dw Syn-DP: 0.0035-0.82 µg/kg dw	Schlabach *et al*. (2011)
	Faroe Islands	Marine sediment from Tórshaven harbour, Klaksvík harbour and Skálafjord, each sampled once in either 2007 or 2009. Anti-DP: 0.44-2.5 µg/kg dw	Schlabach *et al*. (2011)

Compartment	Location	Findings	Reference
		Syn-DP: 0.16-0.93 µg/kg dw	
Storm water	Sweden	4 urban storm waters sampled once in 2009 Total isomers: <0.1–1.2 ng/L (WWTP effluent concentrations ranged from <0.1 to 0.83 ng/L)	Kaj *et al*. (2010)

APPENDIX 6 COMPARISON OF LABORATORY BIOCONCENTRATION DATA BETWEEN SUBSTANCES

Given the uncertainties in the available bioaccumulation data for Dechlorane Plus, the available fish laboratory bioconcentration data for substances that were considered to meet the vB criterion as of May 2015[88] are summarised in this appendix. Comparisons of concentrations actually measured in wildlife have not been included because of the size of the task and variability of use patterns and quantities leading to very different exposures. The data in the following table were collated from agreed regulatory reports produced under REACH. Wet weight whole fish concentrations have been estimated from the cited bioconcentration factors (BCF) and water solubilities unless otherwise stated, and do not take account of lipid content. Polyaromatic hydrocarbons other than anthracene have not been considered for the purpose of this exercise (though could in future). Molar concentrations are presented for comparability between substances.

Substance	**CAS No.**	**Molecular weight, g/mol**	**BCF, L/kg**	**Maximum fish concentration in BCF test**		**Fugacity ratio for the BCF**	**Comment**	**Ref.**
				mg/kg ww	**mmol/kg ww**			
Anthracene	120-12-7	178.2	>6 000	-	-	>2.3	Exposure concentrations are not stated so whole fish concentrations cannot be derived. No information given on depuration half-life.	EC (2008)
Alkanes, C_{10-13}, chloro (short chain chlorinated paraffins)	85535-84-8	371 (assumed: $C_{11}H_{26}Cl_6$)	ca. 7 273	ca. 240	ca. 0.65	0.13	Data are for a C_{10-12} 58 % wt Cl substance based on parent compound analysis. Fish lipid content not stated. Depuration half-lives in the range 39-77 days (for a substance containing 56 % chlorine) and 77-87 days (for a substance containing 69 % chlorine) are reported.	ECHA (2008b)
2-(2H-Benzotriazol-2-yl)-4,6-di-*tert*-pentylphenol (UV-328)	25973-55-1	351.5	4 590	0.37	0.0011	0.01	Based on average BCF at study end. Fish lipid content 4.2 %. No information given on depuration half-life.	ECHA (2014a)

[88] Note: A vB substance with a BCF of 5 000 is only 2.5 times more bioaccumulative than a B substance of similar water solubility with a BCF of 2 000. In the case of another B substance with a BCF of, say, 4 000, it is only 1.25 times higher.

Substance	CAS No.	Molecular weight, g/mol	BCF, L/kg	Maximum fish concentration in BCF test		Fugacity ratio for the BCF	Comment	Ref.
				mg/kg ww	mmol/kg ww			
2-Benzotriazol-2-yl-4,6-di-*tert*-butylphenol (UV-320)	3846-71-7	323.4	9 265	0.93	0.0029	0.12	Fish lipid content 3.6 %. No information given on depuration half-life.	ECHA (2014b)
5-*tert*-Butyl-2,4,6-trinitro-m-xylene (musk xylene)	81-15-2	297.3	3 730 and 10 500	9.89 and 32.7 (estimated)	0.033 and 0.11	1.24 and 3.5	Steady state not reached – plateau fish concentrations were estimated using a one-compartment model. Fish lipid content 3.4 %. Depuration half-life 2.8-4.2 days. Another study resulted in slightly lower fish concentrations (but still >1 mg/kg).	ECHA (2008c)
Hexabromocyclo-dodecane (HBCDD)	25637-99-4	641.7	18 100 and 13 085	112 and 4.45	0.17 and 0.0069	0.76 and 0.55	Fish lipid content not specified. No information given on depuration half-life.	ECHA (2008a)
Henicosafluoro-undecanoic acid	2058-94-8	564.1	ca. 2 700 and 3 700	ca. 1.30 and 0.37	ca. 0.0023 and ca. 0.00066	Not applicable	BCF in first study based on carcass only. Lipid normalisation not appropriate. No information given on depuration half-life.	ECHA (2012b)
Pentacosafluoro-tridecanoic acid	72629-94-8	664.1	ca. 18 000 and ca. 13 000	ca. 3.60 and ca. 1.30	ca. 0.0054 and ca. 0.0020	Not applicable	BCF in first study based on carcass only; second study cited as a BCF range so estimated fish concentration is based on the average. Lipid normalisation not appropriate. No information given on depuration half-life.	ECHA (2012c)
Heptacosafluoro-tetradecanoic acid	376-06-7	714.1	ca. 23 000 and ca. 16 500	ca. 0.32 and ca. 1.65	ca. 0.000045 and ca. 0.0023	Not applicable	BCF in first study based on carcass only; second study cited as a BCF range so estimated fish concentration is based on the average. Lipid normalisation not appropriate. No information given on depuration half-life.	ECHA (2012d)

Substance	CAS No.	Molecular weight, g/mol	BCF, L/kg	Maximum fish concentration in BCF test		Fugacity ratio for the BCF	Comment	Ref.
				mg/kg ww	mmol/kg ww			
Octamethylcyclo-tetrasiloxane (D4)	556-67-2	296.6	≥11 495	≥2.64	≥0.0089	0.05	Fish lipid content 6.4 %. Depuration half-lives reported to be around 3.8 days in one study and 105 days in another.	EA (2009a)
Decamethylcyclo-pentasiloxane (D5)	541-02-6	370.8	≥5 860 and ca. 12 600	≥24.3 and 13.0	≥0.066 and ca. 0.035	0.001 and 0.002	In the first study, fish lipid content varied from 2.9 to 4.1 % during the uptake phase. In the second study, the variation was less and the mean lipid content was 5.71 %. Depuration half-lives of 24-39 days were determined in one study and 19-22 days in another study.	EA (2009b) and EA (2014)
Pentabromo-diphenyl ether (pentaBDE)	32534-81-9	Penta-BDE: 564.7 Hexa-BDE: 643.6	Penta-BDE: ca. 17 700 Hexa-BDE: ca. 5 640	Penta-BDE: ca. 42 Hexa-BDE: ca. 1.37	Penta-BDE: ca. 0.074 Hexa-BDE: ca. 0.0021	0.09 (PentaBDE)	The analysis is complicated because several congeners were tested at the same time, and some corrections have to be made to the data. The cited data are for one pentaBDE and one hexaBDE constituent, respectively. Fish lipid content was 4.8 %. No information given on the depuration half-life.	EC (2001a)

The recalculation of the molar concentration in fish is based on a number of assumptions and consequently has uncertainties. The original references could be reviewed in detail to find out if information on actual fish wet weight concentrations and elimination half-lives is available, and more work could be done to lipid normalize the data (which also involves assumptions). However, this is beyond the scope of this particular dossier. The table should therefore be viewed as provisional.

Whole fish concentrations associated with a high BCF depend on the water solubility achieved in the experiment as well as (usually) the size and lipid content of the test organisms, species-specific factors (such as metabolism, which may change with life stage), and growth dilution, etc. Comparisons between studies using the same substance can therefore be complicated, and comparisons between substances should be treated with caution. Nevertheless, considering only fish BCFs above 5 000 L/kg, these "vB" substances achieved fish concentrations of between 0.000045 and ca. 0.65 mmol/kg ww.

Another possible way to consider the BCF data is in terms of the fugacity ratio. The fugacity ratio for a BCF can be derived approximately using the following approach (Gobas *et al*., 2015).

- The fugacity of a chemical in the water phase $f_W = C_W/Z_W$, where C_W is the concentration in water (mol m^{-3}) and Z_w is the fugacity capacity of the water (mol m^{-3} Pa^{-1}). The value of f_w has units of Pa.

- The fugacity of a chemical in the lipid phase of biota $f_L = C_L/Z_L$, where C_L is the concentration in lipid (mol m^{-3}) and Z_L is the fugacity capacity of the lipid phase (mol m^{-3} Pa^{-1}). The value of f_L has units of Pa.

- The fugacity capacity of lipid is related to the fugacity capacity of water by the following equation: $Z_L = K_{LW} \times Z_w$, where K_{LW} is the water-lipid partition coefficient (dimensionless).

- Assuming that the K_{LW} can be approximated to the octanol-water partition coefficient (K_{OW}), then Z_L approximates to $K_{OW} \times Z_W$ and hence f_L approximates to $C_L/(K_{OW} \times Z_W)$.

- The fugacity ratio for the concentration in fish lipids/concentration in water is therefore $f_L/f_W = C_L/C_W \times K_{OW}$.

- In order to convert the concentrations in fish from a) volume based units to the mass based units used for the BCF and b) from lipid concentrations to whole fish concentrations, it is necessary to use the density of lipid (taken to be 900 kg m^{-3}) and the lipid fraction of the biota. Taking these into account, the fugacity ratio can be written as follows:

    - f_L/f_W = Concentration in fish (mg/kg ww) × density of lipid (0.9 kg/L)/(Concentration in water (mg/L) × fraction of lipids in fish × K_{OW})

    - As the concentration in fish/concentration in water is equivalent to the BCF, the fugacity ratio corresponding to the BCF value can be estimated as BCF × 0.9/(fraction of lipids in fish × K_{OW}). The fugacity ratios estimated for the benchmark chemicals are summarised in the Table. As the perfluorocarboxylic acid compounds are thought not to partition into lipids, this calculation is not appropriate for these substances. Where no lipid content for the fish is given a lipid content of 5 % has been assumed for the calculation.

As with the concentration comparisons, the fugacity ratios also show a high variability (three orders of magnitude).

The high variability in molar concentration (four orders of magnitude) raises an important issue about the use of BCF alone for assessing bioaccumulation potential. In terms of the PBT concept, bioaccumulation concerns are linked to the potential for a substance to reach a

toxic threshold in species that have not been tested in the laboratory. This is related to the number of molecules of a xenobiotic within an organism (available to exert toxicity), and so both water solubility and molecular weight are important considerations alongside the BCF, since it is the molar concentration of a substance in fish tissue that is most relevant. This has not been routinely considered in PBT assessments in the EU up to now.

The WSKOW program within EPISuite (U.S. EPA, 2012) uses the following equation to relate water solubility to the K_{OW}:

$$\log S = 0.693 - 0.0092 (MP - 25) - 0.96 \log K_{OW}$$

where S = solubility in moles/L; MP = melting point in °C

This clearly shows that water solubility (as a molar concentration) generally declines with increasing log K_{OW}. So, whilst an increase in log K_{OW} correlates with an increase in BCF, the equivalent molar fish concentrations (C_{fish}) will not necessarily increase in line with this. In fact, C_{fish} can be higher for more soluble substances with a low log K_{OW}/BCF value than poorly soluble ones with a high K_{OW}/BCF. It is beyond the scope of this report to consider the implications of this in detail using experimental data for actual substances, but a couple of examples for substances that are not considered to be bioaccumulative illustrate the point. The BCF value for ethylbenzene (CAS no. 100-41-4) has been estimated to be around 91 L/kg (EC, 2007). The water solubility of ethylbenzene is 160 mg/L and so it can be estimated that concentrations up to 14 560 mg/kg ww (137 mmol/kg ww) could theoretically be obtained in aquatic organisms. Similarly cumene (CAS no. 98-82-8) has a calculated BCF of 208 L/kg (and a poorly reported experimental BCF of 35.5 L/kg) (EC, 2001b). The water solubility of cumene is 50 mg/L and so fish concentrations of up to around 10 400 mg/kg ww (82 mmol/kg www). These values are well in excess of the values calculated above for the substances with BCF values >5 000 L/kg. Similarly using the log K_{OW} values of ethylbenzene (3.13) and cumene (3.55), it is possible to estimate the approximate fugacity ratio corresponding to the BCF (assuming the BCF values refer to fish with 5 % lipid contents). These are 1.2 for ethylbenzene and 1.1 for cumene (i.e. values very close to 1) and are again within the range of the fugacity ratios estimated for the bioaccumulative chemicals in the Table above.

A measure of bioaccumulation that takes account of molar concentrations and is less dependent on exposure routes (and ideally can be used with monitoring data) would be useful. In the REACH PBT Guidance (ECHA, 2017a), a value of 0.001 mmol/kg ww [0.02 mmol/kg lipid (normalized to a lipid content of 5 %)] has been selected as a level of accumulation that is unlikely to lead to high body burdens (this relationship is derived from considerations related to critical body burdens (CBB) associated with baseline narcosis, as described in Appendix R.11-1 of the guidance). This is divided by a factor of 10 to account for species differences and organ versus body differences. Whilst the CBB concept is usually interpreted as being linked to quantitative risk assessment, it is an existing indicator of a certain level of concern within PBT assessment.

In the case of Dechlorane Plus (with a molecular weight of 653.73 g/mol), this "critical concentration" is equivalent to 0.65 mg/kg ww [13 mg/kg lw] (without the additional factor of 10), or 0.065 mg/kg ww [1.3 mg/kg lw] (with the additional factor of 10).

As described in Section 3.4 of the main report, the following maximum concentrations have been reported for environmental biota samples:

- ~1 mg/kg ww [~95 mg/kg lw] in fish muscle (Wang *et al*., 2015);
- ~1 mg/kg ww [~7 mg/kg lw] in terrestrial bird eggs (Zheng *et al*., 2014b);
- ~0.5 mg/kg ww [~3.8 mg/kg lw] in terrestrial bird liver and muscle (Sun *et al*., 2012; Chen *et al*., 2013b);
- ~3 mg/kg lw in human blood (Zhang *et al*., 2013).

In addition, a concentration of ~1 000 mg/kg lw in liver in Brown Rats *Rattus norvegicus* was achieved under laboratory conditions when dosed at 100 mg/kg/d via oral gavage for 90 days (Li *et al*., 2013a; similar findings have been made by Li *et al*. (2013a) for Common Quail *Coturnix coturnix*). None of the available laboratory fish studies using aqueous exposure are valid and they are confounded by variable exposure concentrations and potential ingestion of particulates and the substance adsorbed to food. Nevertheless, (non-steady state) concentrations up to 8.78 mg/kg ww were observed in Bluegill Sunfish *Lepomis macrochirus* (Boudreau and Rausina, 1973).

Some of these concentrations exceed the critical concentration without the factor of 10, and all exceed the critical concentration with the factor of 10. The accumulation in rat liver exceeds the highest critical concentration by a factor of around 75. On this basis, Dechlorane Plus can clearly achieve concentrations in biota that may induce narcotic toxicity, and so are of concern in a bioaccumulation context.

Depuration half-life is related to the amount of time needed for an organism to clear a chemical once exposure stops – the longer this is, the more likely it might be that an adverse effect could occur. For comparison, the depuration half-lives in fish for the benchmark substances included in the Table above (where available in the source document[89]) indicate that, with one notable exception (musk xylene), substances with high BCF values generally have relatively long depuration half-lives in at least one species, i.e. generally 20 days or above. The depuration half-life for Dechlorane Plus in fish is around 30-40 days for the anti- isomer and 50-70 days for the syn- isomer, and so these values are consistent with other substances that are known to be very bioaccumulative. The long depuration half-life for Dechlorane Plus is therefore a good indicator of a very bioaccumulative substance.

[89] It is probable that other data are available but it is beyond the scope of this analysis to undertake a thorough search for relevant information.

APPENDIX 7 BENCHMARKING OF BIOACCUMULATION USING POLYCHLOROBIPHENYLS (PCBs)

The fish bioaccumulation data summarised in the WHO assessment of PCBs (WHO, 1993)[90] has been considered. Table 6 summarises the section focussing on studies which provide numerical values for uptake or depuration half-life[91]. The data cover a range of PCBs and hence chlorination level, which affects bioaccumulation. The data are mainly for the commercial products, which are a mixture of homologues and congeners. This complicates interpretation of BCF values for specific congeners.

Table 11: Summary of fish bioaccumulation data from WHO (1993) where uptake or depuration data are available

Species	Time to achieve steady-state	Depuration half-life information	Comment	Reference
Sheepshead Minnow (*Cyprinodon variegatus)*	100 days	18% loss of Arochlor 1248 in 28 days; 15% of Arochlor 1260 in 42 days	Arochlor 1248 and 1260	Hansen *et al.* (1975)
Cape Stumpnose (*Rhabdosargus holubi*)	90 days	50 days	Arochlor 1260	De Kock & Lord (1988)
Goldfish (*Carassius auratus*)	Not achieved in 18 days exposure	13 days. 15 mg/kg still present after 70 days	Clophen a50	(Hattula & Karlog, 1973)
Spot (*Leiostomus xanthurus*	14-28 days	Concentrations decreased by 73% in 84 days	Arochlor 1254	Hansen *et al*. (1971)
Rainbow Trout (*Oncorhynchus mykiss*)	-	After 10 weeks reduction of 50% in liver, 100% in gills and 0% in muscle	Clophen (unspecified)	Braun & Meyhofer (1977)
	-	30% loss in 2 weeks, further 6% loss in 126 days (equivalent to t1/2 of 1.6 days and 2.66 years)	Biphasic elimination observed. PCB-52	Guiney *et al*. (1977)
Coho Salmon (*Oncorhynchus kisutch*)	112 – 200 days (depending on conc.)		Dietary exposure. Arochlor 1254	Mayer *et al*. (1977)

[90] WHO. 1993. Polychlorinated Biphenyls and Terphenyls (second edition). Environmental Health Criteria 140, World Health Organization, Geneva

[91] Note that the original data have not been reviewed, and therefore given their age, the data validity should be treated with caution.

Species	Time to achieve steady-state	Depuration half-life information	Comment	Reference
Channel Catfish (*Ictalurus punctatus*)	150 days (lower conc, Arochlor 1232); >150 days (all other exposures)		Dietary exposure. Arochlor 1232, 1248, 1254, and 1260	Mayer *et al*. (1977)
Atlantic Salmon (*Salmo salar*)	30 days (lower conc), >200 days (higher conc)		Dietary exposure. Arochlor 1254	Zitko (1974)

It can be seen that the shortest time to steady state is 14 days, but often steady state took 100 days or more to attain. Half-lives, where available, are a minimum of 13 days. In some instances, bi-phasic elimination is also suggested, with the first stage being considerably more rapid than the second.

The IPCS (1995)[92] review of the POPs cites the following fish aqueous BCF values for PCBs:
- Arochlor 1260: BCF = 120 000 – 270 000 (Fathead Minnows, DeFoe *et al*., 1978)
- Arochlor 1254: BCF = 61 190 (Channel Catfish, Mayer *et al*., 1977)

In the recent SVHC support document for perfluorohexanesulfonate (PFHxS) the following BCF values are cited for PCBs: PCB-52 (2,2',5,5'-tetrachlorobiphenyl, log BCF = 4.84 – 4.9) and PCB-153 (2,2´,4,4´,5,5´-hexachlorobiphenyl) log BCF = 5.0 – 5.3). The most comparable commercial products appear to be Arochlor 1248 or 1254 as these contain significant amounts of the tetra- and penta- homologues[93].

In summary, measured BCF values significantly exceed 5 000 L/kg for Arochlor 1260, 1254 and most likely 1248[94]. Therefore the data in the above table (for time to reach steady state and depuration half-life) provide a degree of benchmarking for substances with BCF values above 5 000 L/kg.

IPCS (1995) reports the following physico-chemical properties for the relevant PCB homologues:
- Tetrachlorobiphenyl: MW = 292.0 g/mol, water solubility = 0.002 mg/L, log K_{OW} = 5.6-6.5
- Pentachlorobiphenyl: MW = 326.4 g/mol, water solubility = 0.004-0.02 mg/L, log K_{OW} = 6.2-6.5
- Hexachlorobiphenyl: MW = 360.9 g/mol, water solubility = 0.0004-0.0007 mg/L, log K_{OW} = 6.7-7.3

Compared to Dechlorane Plus, the PCBs are smaller in size, more water soluble and have lower log K_{OW} values. Therefore the depuration rate constant values should not be directly read-across to provide an indication of exact BCF values, merely that the BCF values may exceed 5 000 L/kg.

[92] A review of selected persistent organic pollutants. Report ref. PCS/95.39. December 1995

[93] Taken from ATSDR toxicological profile (https://www.atsdr.cdc.gov/ToxProfiles/tp.asp?id=142&tid=26): approximate contents of the relevant Arochlors are: 1232 – mixture of mono-, di-, tri-, tetra- and pentachloro with 75% being mono-, di- and tri-; 1248 - mixture of di-, tri-, tetra-, penta- and hexachloro with 97% being tri-, tetra- and penta-; 1254 – mixture tetra-, penta-, hexa- and heptachloro with 86% being penta- and hexa-; 1260 – mixture of penta-, hexa-, hepta- and octachloro with 82% being hexa- and hepta-;

[94] Without a more comprehensive literature search, it is not known whether Arochlor 1232 has BCF values above 5 000 L/kg.

APPENDIX 8 PREDICTED AQUATIC TOXICITY OF SELECTED DECHLORANE PLUS ANALOGUES USING EPISUITE V4.11

The following table lists the four substances that are considered to be the closest structural analogues to Dechlorane Plus (as discussed in Section 1.4 of the main report). Where available, experimental data for physico-chemical endpoints (such as log K_{OW}) have been used to generate predictions (this applies to chlordane and heptachlor). The ECOSAR model provides results for the vinyl/allyl halides class and the standard neutral organics class. Both are included for comparison. Predicted data are rounded to one or two significant figures.

		Chlordane	**Heptachlor**	**Dechlorane 603**	**Chlordene Plus**
Molecular weight, g/mole		409.78	373.32	637.69	611.61
Water solubility, mg/L at 25 °C	**Experimental**	0.056	0.18	-	-
	WSKOW v1.42	0.02	0.04	2E-8	6E-7
	WATERNT v1.01	0.004	0.009	6E-7	6E-7
Log K_{OW}	**Experimental**	6.1-6.2	5.5-6.1	-	-
	KOWIN v1.68	6.3	5.9	11.2	9.8
ECOSAR v1.11, vinyl/allyl halides[95], mg/L	**Fish 96-h LC_{50}**	0.011	0.022	5.3E-7*	9.6E-6*
	Daphnid 48-h LC_{50}	0.012	0.023	8.0E-7*	1.3E-5*
	Algae 96-h EC_{50}	0.056*^	0.10^	1.6E-5*^	1.8E-4*^
	Fish ChV	2.5E-4	6.9E-4	4.4E-10	2.1E-8
	Daphnid ChV	0.016	0.02	6.0E-4*	0.0017*
	Algae ChV	0.084*^	0.13^	2.2E-4*^	0.0013*^
ECOSAR v1.11, neutral organic[96], mg/L	**Fish 96-h LC_{50}**	0.05	0.10	2.8E-6*	5.0E-5*
	Daphnid 48-h LC_{50}	0.042	0.084	3.8E-6*	5.8E-5*
	Algae 96-h EC_{50}	0.16*	0.27*	9.4E-5*	8.5E-4*
	Fish ChV	0.008	0.015	7.5E-7*	1.1E-5*
	Daphnid ChV	0.012	0.022	3.9E-6*	4.2E-5*
	Algae ChV	0.10	0.16	1.6E-4*	0.0011*
Harmonized classification under the CLP Regulation		Acute Tox. 4 Carc. 2 Aq. Acute 1 Aq. Chronic 1	Acute Tox. 3 Carc. 2 STOT RE 2 Aq. Acute 1 Aq. Chronic 1	-	-

NOTES:

- ChV (Chronic Value) is the geometric mean of the no observed effect concentration (NOEC) and the lowest observed effect concentration (LOEC). This can be mathematically represented as: ChV =

[95] Maximum log K_{OW} cut-off: 6.0 (Fish and Daphnid LC_{50}), 6.4 (Green Algae EC_{50}) and 8.0 (ChV).

[96] Maximum log K_{OW} cut-off: 5.0 (Fish and Daphnid LC_{50}), 6.4 (Green Algae EC_{50}) and 8.0 (ChV).

10^([log (LOEC x NOEC)]/2). NOEC values can be derived from predicted ChV values by dividing by √2.
- * Indicates that the chemical may not be soluble enough to measure this predicted effect.
- ^ Insufficient data are available to generate a SAR so predictions for this endpoint are based on the acute-to-chronic ratio, and the acute SAR for vinyl/allyl halides.

The vinyl/allyl halides class model is mainly based on chlorinated substances with log K_{OW} values below 5. Heptachlor is included in the training set of the acute fish/daphnia QSAR[97], and chlordane is included in the training set of the chronic fish/daphnia QSAR[98] (no other substances with the halogenated norbornene moiety are included). There are no relevant training set data for the chronic algal endpoint so this is based on an acute-to-chronic ratio using the acute algal SAR endpoint which in turn is based on a single data point. This means the algal endpoints in this model are the least reliable.

The chlordane and heptachlor endpoints are not used in the training set for the neutral organics model. There are substances with a non-halogenated norbornene structure (norbornene (CAS no. 498-66-8) and exo-norborneol (CAS no. 497-37-0)) in the acute fish data training set but they have a much lower molecular weight (<112 g/mol) and log K_{OW} (<3) than the substances being considered.

The two pesticide analogues (chlordane and heptachlor) are known to be toxic, but are not close structural analogues to Dechlorane Plus and are much less hydrophobic. They therefore cannot be used for direct read across purposes, but give an indication of the potential toxicity of this type of substance. They have log K_{OW} values at the upper end of the prediction domain of the models (exceeding that for the baseline fish and Daphnid acute models by about an order of magnitude; chlordane is outside the domain for the vinyl/allyl halides class prediction for fish and Daphnid acute toxicity too). In addition, as noted above, heptachlor and chlordane are part of the training sets for the vinyl/allyl halides model. Consequently, it is not surprising that the predictions for chlordane and heptachlor are in good agreement with measured data. It is beyond the scope of this document to fully summarize additional ecotoxicity data for these pesticides, but both have several fish and invertebrate acute $L(E)C_{50}$ values below 0.01 mg/L (UNEP, 1998; WHO, 1984a&c & 1995). Chronic NOECs will be lower still. It can be concluded that both substances are chronically toxic to fish and aquatic invertebrates, with NOECs below the Annex XIII T criterion threshold of 0.01 mg/L.

With the exception of the chronic fish category, both ecotoxicity models consistently predict that short- and long-term toxicity to aquatic organisms is unlikely to be expressed for the two closest analogues to Dechlorane Plus (Dechlorane 603 and Chlordene Plus), since relevant toxicity endpoints exceed the predicted level of water solubility. Converting the fish ChV endpoints to a NOEC brings that endpoint to within a factor of 10 of the very low solubility predictions, implying that toxicity might be expressed around the level of the water solubility limit. However, both of these substances have log K_{OW} values that exceed the cut-offs for the models by more than an order of magnitude, so these predictions in the view of the DS cannot be considered reliable (i.e. no effects at saturation are expected for these endpoints).

It should be noted that these predictions do not allow any comparison to be made about likely fish toxicity arising from dietary exposure, or mammalian/avian toxicity.

[97] Providing three of forty-one data points in the acute fish SAR (96-h LC_{50} of 0.007 – 0.023 mg/L) and one of eleven data points in the acute Daphnia SAR (48-h EC_{50} of 0.042 mg/L).

[98] Providing one of seven data points for the chronic fish SAR (ChV of 0.0016 mg/L) and one of two data points for the chronic Daphnia SAR (ChV of 0.016 mg/L). The reference for the Daphnia result is dated 1979, so it may not conform to modern standards.

APPENDIX 9 FURTHER REFERENCES THAT APPEAR RELEVANT BUT ARE NOT INCLUDED IN THE ASSESSMENT

The abstracts of the following references have been scanned in order to assess if the paper contains information that may be relevant, but they are considered unlikely to significantly affect the conclusions of the assessment.

Barón E, Bosch C, Máñez M, Andreu A, Sergio F, Hiraldo F, Eljarrat E and Barceló D (2015). Temporal trends in classical and alternative flame retardants in bird eggs from Doñana Natural Space and surrounding areas (south-western Spain) between 1999 and 2013. Chemosphere, 2015, 138, 316-323.
Relevance: Temporal trends in concentrations in birds' eggs from Spain. No time trend appears to have been observed for DP.

Dou J, Jin Y, Li Y, Wu B and Li M (2015). Potential genotoxicity and risk assessment of a chlorinated flame retardant, Dechlorane Plus. Chemosphere, 135, 462-466.
Relevance: Human health, including short-term toxicity to bacteria. This study is mentioned in the registration dossier, although is not directly relevant to the PBT assessment.

Fromme H, Cequier E, Kim JT, Hanssen L, Hilger B, Thomsen C, Chang YS and Völkel W (2015). Persistent and emerging pollutants in the blood of German adults: Occurrence of dechloranes, polychlorinated naphthalenes, and siloxanes. Environment International, 85, 292-8.
Relevance: Levels in human blood from Germany. The median level found in 42 plasma samples were 1.23 ng/g lipid for anti-DP and 0.77 ng/g lipid for syn-DP.

Kakimoto K, Nagayoshi H, Takagi S, Akutsu K, Konishi Y, Kajimura K, Hayakawa K and Toriba A (2014). Inhalation and dietary exposure to Dechlorane Plus and polybrominated diphenyl ethers in Osaka, Japan. Ecotoxicology and Environmental Safety, 99, 69-73.
Relevance: Concentrations in human foodstuffs (Japan). The Registrant has summarized this under environmental monitoring – RSS #17.

Kim J, Son M-H, Kim J, Suh J, Kang Y and Chang Y-S (2014). Assessment of Dechlorane compounds in foodstuffs obtained from retail markets and estimates of dietary intake in Korean population. Journal of Hazardous Materials, 275, 19–25.
Relevance: Concentrations in human foodstuffs (Korea). The Registrant has summarized this under environmental monitoring – RSS #18.

Liu L-Y, Salamova A and Hites RA (2014). Halogenated flame retardants in baby food from the United States and from China and the estimated dietary intakes by infants. Environmental Science and Technology, 48 (16), 9812-9818.
Relevance: Concentrations in human foodstuffs (North America and China).

L'Homme B, Calaprice C, Calvano CD, Zambonin C, Leardi R and Focant JF (2015). Ultra-trace measurement of Dechloranes to investigate food as a route of human exposure. Chemosphere, 139, 525-533.
Relevance: Levels in human food items in Belgium.

Möller A, Xie Z, Caba A, Sturm R and Ebinghaus R (2012). Occurrence and air-seawater exchange of brominated flame retardants and Dechlorane Plus in the North Sea. Atmospheric Environment, 46, 346-353.
Relevance: Levels in air and seawater in the German part of the North Sea in 2010. The concentration of dechlorane plus ranged from 0.13 to 22.3 pg/m^3 in air and from 0.10 to 17.7 pg/L in air and seawater. The highest levels in air were found in air masses influenced by the continent and the highest levels in seawater were observed at sites close to the coast/influenced by riverine discharge. Concluded that, besides riverine discharge, both dry air-seawater gas exchange and dry deposition are input pathways for dechlorane plus in the North Sea.

Sühring R, Busch F, Fricke N, Kötke D, Wolschke H and Ebinghaus R (2016). Distribution of brominated flame retardants and dechloranes between sediments and benthic fish — A comparison of a freshwater and marine habitat. Science of the Total Environment, 542, 578–585.
Relevance: Concentrations in marine and freshwater sediments and fish in Germany.

Sun R-X, Luo X-J, Tan X-X, Tang B, Li Z-R and Mai B-X (2015). An eight year (2005-2013) temporal trend of halogenated organic pollutants in fish from the Pearl River Estuary, South China. Marine Pollution Bulletin, 93 (1-2), 61-67.
Relevance: Concentrations in fish near to sources of release, outside Europe. No clear temporal trend was evident for DP.

Sun R-X, Luo X-J, Tan X-X, Tang B, Li Z-R and Mai B-X (2015). Legacy and emerging halogenated organic pollutants in marine organisms from the Pearl River Estuary, South China. Chemosphere, 139, 565-571.
Relevance: Concentrations in fish near to sources of release, outside Europe.

Tao W, Zhou Z, Shen L and Zhao B (2015). Determination of dechlorane flame retardants in soil and fish at Guiyu, an electronic waste recycling site in south China. Environmental Pollution, 206, 361-368.
Relevance: Levels in fish associated with a source of emission, outside Europe. [Photodegradation results are included in this report.]

Vetter W, Gall V and Skírnisson K (2015). Polyhalogenated compounds (PCBs, chlordanes, HCB and BFRs) in four polar bears (*Ursus maritimus*) that swam malnourished from East Greenland to Iceland. Science of the Total Environment, 533, 290-296.
Relevance: Occurrence in biota. Abstract suggests that Dechlorane 602 was tentatively identified but no information is given in the abstract on DP.

Wang P, Zhang Q, Zhang H, Wang T, Sun H, Zheng S, Li Y, Liang Y and Jiang G (2016). Sources and environmental behaviors of Dechlorane Plus and related compounds — A review. Environment International, 88, 206–220.
Relevance: Review article.

Wellington Science Associates, Inc. (1978). The Characterization of the Diels-Alder Adducts of Hexachlorocyclopentadiene and Assessment of their Use. Fish. Environ. Can. MS Rep. No. O.R.2O.
Relevance: Summary of chemical properties and historical use patterns of Dechloranes. Original reference not located.

Zhang H, Bayen S and Kelly BC (2015). Co-extraction and simultaneous determination of multi-class hydrophobic organic contaminants in marine sediments and biota using GC-EI-MS/MS and LC-ESI-MS/MS. Talanta, 143, 7-18.
Relevance: Analytical method development for concentrations in biota, outside Europe.

Zhou SN, Reiner EJ, Marvin C, Kolic T, Riddell N, Helm P, Dorman F, Misselwitz M and Brindle ID (2010). Liquid chromatography–atmospheric pressure photoionization tandem mass spectrometry for analysis of 36 halogenated flame retardants in fish. J Chromatogr A, 1217, 633–641.
Relevance: Concentrations in fish, sampling area/relevance unclear from the abstract.

Zhou SNS, Reiner EJ, Marvin CH, Helm PA, Shen L and Brindle ID (2011). Liquid chromatography/atmospheric pressure photoionization tandem mass spectrometry for analysis of Dechloranes. Rapid Commun. Mass Spectrom. 2011, 25 (3), 436−442.
Relevance: Concentrations in fish, sampling area/relevance unclear from the abstract (may contain similar data to the Zhou et al. (2010) paper above).