MCCP REACH Consortium

Statement for ECHA PBT Expert Group Review of MCCP
August 2019

Overview

Alkanes, C14-17, chloro, (EC 287-477-0) also known as medium-chain chlorinated paraffin (MCCP) is a complex (UVCB) substance derived from the chlorination of n-alkane (i.e. paraffin) feedstocks. Some key considerations for the PBT evaluation of MCCP are:

1) The chlorination process is random, resulting in substance that has 10 000+ isomers. This complexity impacts chemical analysis. It is impossible to identify individual chemical constituents, though advanced analytical techniques can separate and quantify constituents by congener groups that have the same molecular weight (e.g. $C_{14}H_{26}Cl_4$).

2) MCCP is very poorly soluble in water. Latest study showed a mean of 6 µg/L (±29%) for a C14, 50% chlorination by weight, with solubility levels dropping considerably for more chlorinated congener groups. Water solubility estimates for MCCP are generally in the range of 4-10 µg/L.

3) MCCP is poorly absorbed via diet. Assimilation efficiency (whole fish) was 6.7% for C14, 50% Cl by wt., whereas the assimilation efficiency was 82.5% for hexachlorobenzene (HCB) in the same OECD 305 fish dietary bioaccumulation study. The assimilation efficiency results from the new OECD 305 fish dietary study are similar to earlier fish accumulation studies, as where the depuration rates.

4) MCCP biodegradation/biotransformation appears to be greatly impacted by bioavailability. Considerable biodegradation/biotransformation was observed in OECD 301D biodegradation studies, with limited organic content in the test system, though little biotransformation was observed in OECD 308 sediment studies with higher organic content.

5) The European Food Safety Authority (EFSA) has recently completed a draft "Scientific opinion on the risk for animal and human health related to the presence of chlorinated paraffins in feed and food" which assessed the potential risks from MCCP exposure via dietary sources. Its main conclusions were that the risks from MCCP exposure via the diet in the EU appears to be very low (more than several orders of magnitude below the health benchmark).

6) Recent environmental monitoring levels in European sediment and water are below PNECs and field B studies general show that MCCP does not appear to biomagnify.

7) The registrants currently warn against releases to water and that wastes must be handled properly.

P and B Endpoint Summaries from MCCP Chemical Safety Report

Biodegradation

The biodegradation of MCCP has been evaluated by numerous studies on both commercial products and specifically identified components of interest, in particular different chlorinated versions of C14(tetradecane) and C15(pentadecane). The ability to test the biodegradation of MCCP is highly dependent upon the testing system set up given the very low water solubility of MCCP and its high potential to sorb onto organic matter and vessel walls in the test system. Studies that have taken this into account and made MCCP more bioavailable have demonstrated that MCCP up to 50% chlorination by weight is readily biodegradable and that MCCP in the 50-60% chlorination (by wt.) is inherently biodegradable.

A series of GLP Closed Bottle Test (CBT), OECD 301D guideline, studies were conducted in 2009 and 2010 to evaluate the ready biodegradation of MCCP and its constituents (van Ginkel, 2010 a,b,c,d,e). In these CBT studies 2 mg/L of the test material was incubated in the dark with either river water or secondary activated sludge derived from a plant treating predominantly domestic wastewater and oxygen consumption was measured by analyzing for dissolved oxygen content. These studies were conducted using procedures to improve the bioavailability of the test material to the inoculum, in accordance with the guideline. In the first study, a chlorinated tetradecane (C14) at 45% Cl (wt.) showed biodegradation on days 0, 7, 14, 21, 28 and 42 of 0, 3, 14, 32, 64, and 67%, respectively. Therefore, this test material is considered "readily biodegradable" (van Ginkel, 1). Subsequently, three MCCP commercial products (46.5, 51.7% and 63.2% Cl wt.) were assessed in the CBT (van Ginkel, 2010, b,c,d). Lastly, a comparative study of the biodegradation rates of chlorinated paraffins by chlorination levels was conducted using tetradecane chlorinated across the range from approximately 40% to 60% Cl (wt.) (van Ginkel 2010e). Table 1 below summarizes the results of these studies.

Table 1: Percent Degradation by Test Material from van Ginkel 2010 CBT Studies

Test Material	C14, 45% Cl(a)	MCCP, 45.6% Cl(b)	MCCP, 51.7% Cl(d)	MCCP, 63.2% Cl(c)	C14, 41.4% Cl(e)	C14, 45.4% Cl(e)	C14, 50.0% Cl(e)	C14, 55.0% Cl(e)	C14, 60.2% Cl(e)
Sampling Day									
7	3	0	7	0	3	5	1	0	4
14	14	9	10	5	34	28	13	4	11
21	32	31	17	14	56	64	46	18	22
28	64	51	27	5	62	73	54	30	28
42	67	63	47	5	74	75	71	50	39
56					83	73	78	57	49
60			57	10					

a: van Ginkel 2010a
b: van Ginkel 2010b
c: van Ginkel 2010c
d: van Ginkel 2010d
e: van Ginkel 2010d

Additional OECD guideline 301D CBT studies (van Ginkel 2014a,b and van Ginkel 2018a,b,c,d) were conducted on polychlorinated tetradecane (C14, 50, 55, 60% Cl by

wt.) and pentadecane (C15, 51% Cl by wt.) as these test substances were identified by ECHA and the U.K. Environment Agency for additional evaluation as a part of an overall substance evaluation of MCCP. The 2014 studies on C15 (51% Cl wt.) found that the test material degraded up to 63% on day 60, indicating that the test material is inherently biodegradable and not persistence. The 2018 CBT studies included additional chemical analysis of the test materials beyond the standard biodegradation parameter, oxygen consumption in the case of the OECD 301D, and were conducted over an extended period of 120 days. The chemical analysis portion of this testing, conducted by Vrije Universitat (VU) Amsterdam, compared two different high-resolution methods of chemical analysis – GCxGC-ECD and APCI-TOFMS - to determine their abilities to measure and distinguish chloroalkane congener groups (e.g. $C_{14}H_{26}Cl_4$) within the test materials. Both methods performed well and were able to quantify C14congener groups from $Cl_4$to Cl_{11}, which represent a range of approximately 35-68% chlorine by weight. This is the first time that such a congener group analysis has been applied to the biodegradation testing of MCCP. It should be noted that whilst the test materials are chlorinated to specific levels of chlorine by weight, they all contain generally the same congener groups just in different proportions. The removal of the parent compounds after 120 days by congener group for the chlorinated tetradecane studies (van Ginkel 2018a,b,c) are provided in the table below, including a comparison of the two analytical methods used, except in the case of the C14, 60% Cl test material where the results of a second experiment were included given the significant removal results from the first experiment.

Table 2: Removal of Chlorinated Congener Group in CBT Studies (van Ginkel 2018a-d) After 120 Days

Table 2: Removal of Chlorinated Congener Group in CBT Studies (van Ginkel 2018a-d) After 120 Days

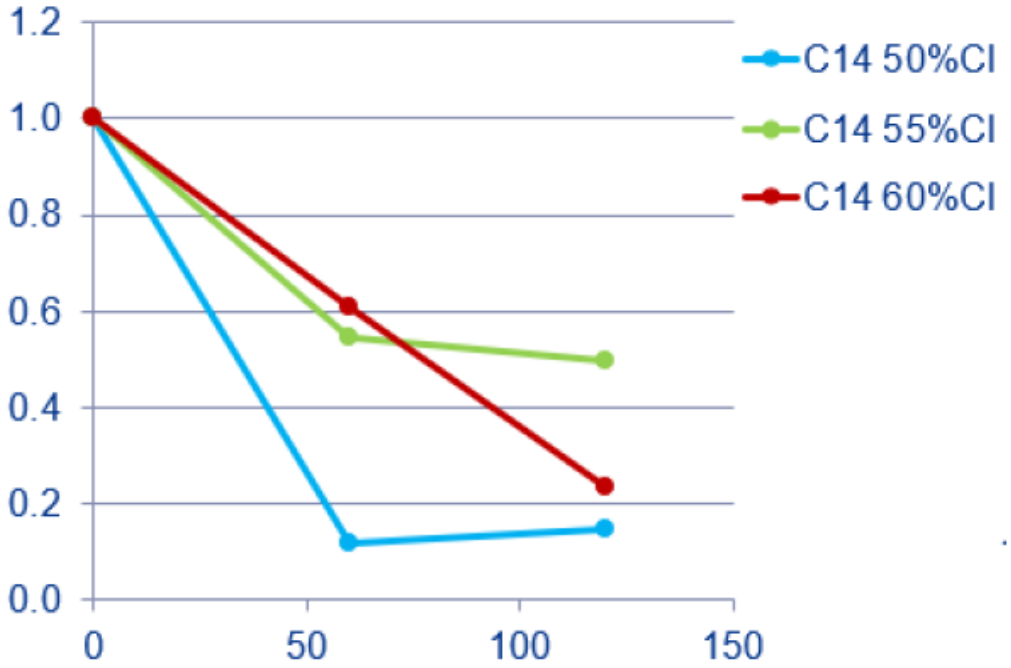
Test Material:	C14 50%Cl		C14 55%Cl		C14 60%Cl		C15 51%Cl	
Congener Group	GCxGC-ECD	APCI-TOFMS	GCxGC-ECD	APCI-TOFMS	APCI-TOFMS 1st Experiment	APCI-TOFMS - 2nd Experiment*	GCxGC-ECD	APCI-TOFMS
Cl_4	92%	99%	74%	76%	92%	86%	93%	-**
Cl_5	92%	97%	74%	71%	92%	82%	89%	96%
Cl_6	90%	93%	75%	68%	92%	81%	89%	95%
Cl_7	83%	87%	61%	62%	91%	80%	84%	88%
Cl_8	83%	75%	64%	48%	91%	78%	78%	76%
Cl_9	83%	60%	57%	19%	89%	76%	76%	66%
Cl_{10}	73%	57%	46%	-	86%	70%	72%	61%
Cl_{11}	79%	71%	49%	-	80%	60%	65%	56%
Cl_{12}	76%	82%	27%	-	75%	50%	41%	52%
Total reduction	86%	85%	65%	50%	89%	77%	83%	86%

* A second experiment was conducted and analyzed given the greater than expected removals in the first experiment.
** Reliable results were not determined for this congener group using the APCI-TOFMS

method.

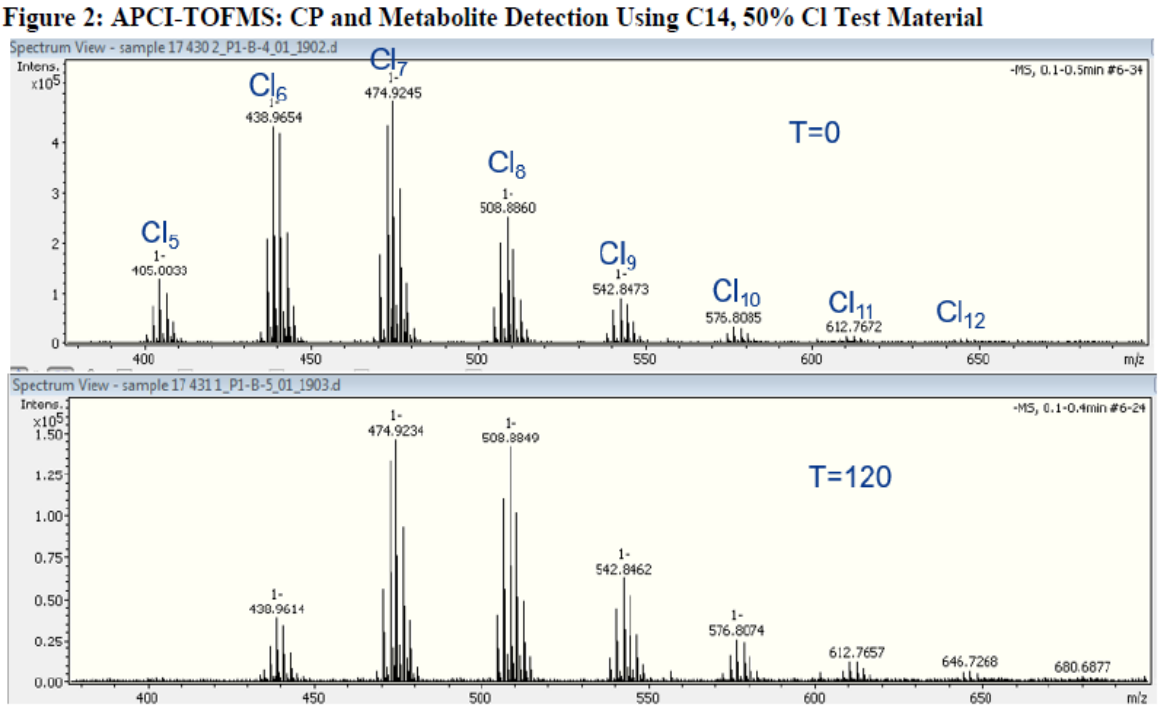
For the C14, 50% and 55% Cl (by wt.) test materials the Day 60 results are very similar to the Day 120 results (see Figure 1 below). For the C14, 60% Cl (by wt.) test material there continued to be significant biodegradation from Day 60 to Day 120. These results may be indicative of the slower biodegradation rates of more highly chlorinated congener groups, which will have more highly chlorinated metabolites. The more extensive biodegradation of the C14, 60% Cl (by wt.) test material compared to the C14, 55% Cl (by wt.) was an unexpected result, though a second experiment confirmed the extensive biodegradation of this test material.

Figure 1: Test Material Removal Rates for C14 Test Materials in Closed Bottle Tests (van Ginkel 2018a-c)



An attempt was made to analyse for metabolites in these CBT studies. Whilst metabolites were detected, their specific identification and quantification is difficult without having established standards. The APCI-TOFMS method was felt to be superior for metabolite detection since the metabolite and CP could still be clustered into congener groups. Figure 2 shows the Day 0 and Day 120 mass spectrometry analysis of the C_{14}, 50% Cl test material. These results suggest that essentially all of the Cl_4, Cl_5 and most of the Cl_6 test material and metabolites are completely mineralized but that there will be metabolites and CP test material remaining from Cl_7 and up. The fact that the Cl_7 peaks are about the same and Cl_8 and Cl_9 peaks are higher is being interpreted as a sign of the formation of metabolites in these congener groups from higher (i.e. Cl_{10}+) congener groups in the test material.

Figure 2: APCI-TOFMS: CP and Metabolite Detection Using C14, 50% Cl Test Material



In earlier studies, the degradation of four $C_{14\text{-}17}$ chlorinated paraffins (with extent of chlorination ranging from 40 to 58%) has been determined by measuring oxygen consumption during incubation with non-acclimated microorganisms for up to 25 days at concentrations of 2, 10 and 20 mg/L. The extent of biodegradation varied from 15% (for the 40% chlorinated paraffin) to 0% (for the 58% chlorinated paraffin) at day 25. The 5-day biochemical oxygen demand (BOD5) values ranged from 0.02 g O2/g of a 40% chlorinated C14-17 paraffin to "no significant oxygen uptake" for a 58% chlorinated $C_{14\text{-}17}$ paraffin (Madeley and Birtley, 1980).

The degradation of two MCCPs has been studied by Omori et al. (1987) using a variety of bacterial cultures. The MCCPs studied had averages of $C_{14.5}$ (43.5% chlorinated) and $C_{15.4}$ (50% chlorinated) and degradation was studied by monitoring the release of chloride ion. Firstly, degradation of the 50% chlorinated paraffin was studied using resting cell cultures of Pseudomonas aeroginosa, Achromobacter delmarvae, A. cycloclastes, Micrococcus sp. and Corynebacterium hydrocarboclastus grown on glycerol. Little or no degradation (chloride release) was observed when the C_{15} chlorinated paraffin was incubated with the bacteria for 24 hours at 30°C. In addition, cometabolic biodegradation experiments were carried out with the $C_{14.5}$ and the $C_{15.4}$ chlorinated paraffins (43.5% and 50% chlorinated) at a concentration equivalent to 180 mg Cl/1.2 L solution, using a mixed bacterial inoculum (containing strains HK-3, HK-6, HK-8 and HK-10) incubated at 30oC for 48 hours. Bacterial strains were isolated from soil (no further details on soil characteristics given in paper) using an enrichment culture containing n-hexadecane as the sole carbon source. Both paraffins showed significant degradation, with 77 mg/L and 85 mg/L of chloride being released from the 50% and 43.5% chlorinated paraffins, respectively, after 36 hours. As the starting MCCP concentration was 180 mg Cl/1.2 L solution, about 51 and 57% of the chlorine present was released during the degradation, respectively. These findings suggest the potential for

biodegradation appears to increase with decreasing chlorine content (Omori et al. 1987). The authors concluded that the degradation seen was consistent with that previously observed for other chlorinated alkanes in that a variety of enzymes are required to degrade chlorinated paraffins and that the most likely mode of degradation involves firstly dechlorination of the terminal methyl groups, with subsequent oxidation to form chlorinated fatty acids, which are then broken down to 2- or 3-chlorinated fatty acids via β-oxidation (EU, 2005).

A recent analysis of the likely biochemical biodegradation pathways was conducted on a series of theoretical constituents in MCCP (Federle 2017). This assessment identified multiple biodegradation pathway options that may exist for a chlorinated paraffin (CP) molecule and that all of these pathway options involve conversion of the CP to a chlorinated fatty acid, shortening of the carbon chain and dechlorination. Each CP isomer will be degraded by a somewhat different pathway but overall these pathways lead to increasingly polar and less toxic metabolites. Pathway options decline as chlorination levels and occurrence of vicinal chlorine substitutions increase (Federle 2017 – attached in Section 13.2 of the dossier). One important conclusion from this work is that MCCP constituents cannot degrade into SCCP constituents as the chain-shortening reaction will involve oxidation of the molecule to a fatty acid.

The half-lives of C_{16} chlorinated paraffins were estimated at about 12 days (35% chlorinated) and 58 days (69% chlorinated), respectively, in an aerobic sediment system (from a freshwater lake) containing oligochaete worms (Lumbriculus variegatus). The extent of degradation was determined at day 0 and day 14 of the experiments based on the difference between toluene-extractable ^{14}C measurements (taken to represent unchanged chlorinated paraffin) and total ^{14}C measurements (Fisk et al. 1998). These substances represent the extremes of the typical chlorine contents of MCCPs in commercial production. The RAR (EU, 2005) notes that the "results of this test should be treated with caution as the identity of the 14C present in the samples was not determined, and it was only assumed that the non-extractable 14C represented metabolites". A new OECD 308 sediment simulation study was conducted at EAG Laboratories with analysis by Vrije Universitat (VU) Amsterdam using the same analytical methods used in the CBT studies (van Ginkel 2018a-d). The test was conducted using two sediments and their associated waters. Test systems were dosed with C14, 50% Cl (by wt.) test material at a nominal concentration of 5 µg/g dry weight of sediment. Test systems were housed on a shaker table in an incubator set to maintain a temperature of 12 ºC for up to 120 days. The initial results from this study are inconclusive as the recoveries of the test material from the sediment have varied greatly, likely due to the interference of the organic matter in the sediment. Additional analysis is ongoing, but there are currently no conclusions from this study.

Adsorption onto sludge is likely to be the major removal mechanism for MCCPs during waste water treatment processes. A removal rate of 97.1% by adsorption onto sewage sludge during waste water treatment was obtained using the SIMPLE TREAT 4.0 model on the influent values reported by Coelhan 2010 (see analysis in CSR).

There is clear evidence that microorganisms are capable of degrading MCCPs in the environment based on the extensive biodegradation observed in the CBTs and that the rate of biodegradation decreases with increasing chlorine content. However, given the range of chlorination levels in this assessment a worst-case approach of no biodegradation was assumed in the PEC calculations.

Hydrolysis is not expected to be a significant degradation process for MCCPs in the environment. An atmospheric half-life of 1-2 days is estimated for MCCPs for reaction with hydroxyl radicals. A value for the rate constant for the reaction (kOH) of 8.10-12 cm^3 molecule-1 s-1 will be used for the environmental modelling in the risk assessment (EU, 2005).

Bioaccumulation

Bioaccumulation is broadly defined as a process by which the concentration of a chemical in an organism exceeds that in the respiratory medium (e.g. water for fish, air for mammals), in the diet, or both (Gobas et al, 2009). Highly bioaccumulative chemicals are perceived as problematic because harmful (toxic) concentrations may be achieved in organisms, including humans, at the top end of food chains, even though the 'source' (e.g. water) concentrations are not directly toxic. Although the various regulatory schemes attempt to set both quantitative and qualitative criteria to define the level of bioaccumulation deemed hazardous, interpretation of these criteria in relation to the different types of scientific data that are, or are not, available for a particular chemical is often difficult. In response to this difficulty, Gobas et al (2009), as part of SETAC Pellston Workshop in 2008 considering all factors relevant to the identification of PBTs and POPs, reviewed the state of the science and proposed a framework for the evaluation of bioaccumulation in relation to the regulatory situation.

In the case of medium-chain chlorinated paraffin (MCCP), alkanes, $C_{14\text{-}17}$, chloro, there are a variety of data available to assess the bioaccumulation potential, including:

- Octanol-water partition coefficient (KOW), both estimated and measured;
- Bioconcentration factor (BCF) as measured in laboratory tests;
- Bioaccumulation factor (BAF) as measured in laboratory tests and field studies;
- Biota-Sediment Accumulation Factor (BSAF) as measured in laboratory tests;
- Biomagnification factor (BMF) as measured in laboratory tests and field studies; and
- Trophic magnification factor (TMF) as measured in field studies.

By definition KOW, BCF, BAF, BSAF, BMF and TMF are steady-state metrics, i.e., there are no significant changes in chemical concentrations over time. K_{OW} is used as a surrogate for lipid-water equilibrium partitioning and has recognized limitations for B assessment, primarily because it is only a chemical property and ignores biological processes such as biomagnification and biotransformation (Arnot 2008, Nichols 2007, Arnot 2013). The BCF is the ratio of the chemical concentration in a fish to the chemical concentration in the water following chemical exposure from the water only. The BCF is measured under controlled laboratory conditions; there is no dietary exposure. The BAF is the ratio of the chemical concentration in a fish to the chemical concentration in the water as a result of all routes of exposure (i.e. water and food). The BMF is the ratio of the chemical concentration in an organism to the chemical concentration in its diet. The BMF can be determined through laboratory (model) testingorfield measurements. Field BMFs include all routes of exposure, whereas laboratory BMFs only include dietary exposures under controlled conditions in which there is no exposure to chemical in the water. The TMF is the average factor by which the chemical concentration in biota of a food web increases per trophic level and is determined from environmental monitoring data, i.e., organisms are exposed to chemical from the environment and diet. Obtaining accurate BCFs and BAFs for very hydrophobic chemicals like MCCP constituents is challenging because of technical difficulties and a general lack of extensive scientific knowledge on the actual dissolved (bioavailable) chemical concentration in the water for such hydrophobic ("water-hating") chemicals. Rationales for lipid correction (normalizing) for neutral organic chemicals and growth correction of the measured data are provided elsewhere (Gobas 2009, Burkhard 2012, Arnot 2013, Connolly 1988, Mackay 1982, OECD. 2012).

Growth Correction of Bioaccumulation Results

The issue of growth correction for BCF values has been raised by reviewers in the context of the bioaccumulation assessment of MCCP given the potential concern for growth dilution in laboratory studies. Historically growth correction has not been applied

in B assessments (i.e., pre-2012) and it is unclear how these corrections are to be evaluated against historical BCF standards which themselves are based on non-growth corrected data. Laboratory bioaccumulation studies are typically started with young fish so limiting growth is almost an impossibility. In the interest of being responsive and transparent on this issue, growth corrected values have been provided on key studies where the data permit (e.g. Thompson 2000 and Hurd and Vaughan 2010), though caution should be applied in their interpretation. This is perhaps yet another reason to view the broad array of data available to assess the bioaccumulation potential of MCCP as opposed to any one study or specific type of data.

Octanol-Water Partition Coefficient Data

Measurements and predictions for the octanol-water partition coefficient (K_{OW}) for MCCP constituents span a few orders of magnitude (log K_{OW}s from ~5.5 to >9.0). These very high K^{OW}s reflect the fact that MCCP constituents are very hydrophobic ("water-hating") and hence readily partition from water to other phases.

Bioconcentration Factor Data

There are 2 existing GLP guideline laboratory bioaccumulation studies on MCCP related test materials in fish (Thompson 2000 and Hurd and Vaughan 2010) that reported bioconcentration factor (BCF) results. Only Thompson (2000) was conducted on a full range MCCP test material at approximately 51% Cl by weight and this study also employed a radiolabelled pentadecane (C15) at approximately 51% Cl by weight. Several published dietary fish studies are also available on chlorinated C_{14} and C_{16} test materials (Fisk 1996, 1999, 2000) and one in earthworms using a C_{16} chlorinated test material (Fisk 1998). An additional earthworm toxicity study (Thompson 2001) on a full-range MCCP product at 52% chlorination by weight also provided some estimates of bioaccumulation, though these results have limitations including high test material concentrations. A new dietary bioaccumulation study in fish according to the OECD guideline 305 on a chlorinated tetradecane (C14) at 50% chlorine by weight is in progress and should be completed in 2018. This study will be the first to include analysis of individual chloroalkane congener groups.

Thompson (2000) is a GLP OECD guideline 305 study in rainbow trout (Oncorhynchus mykiss) using full range MCCP and a radiolabelled n-pentadecane-8-^{14}C (both at 51% chlorine by weight). The bioconcentration factor (BCF) based on a kinetic estimate was 1087 and 349 at a nominal exposure concentration of 1 and 5 µg/L, respectively, at day 35. The kinetic estimates of time for tissue concentrations to reach 95% steady state levels were 67 and 74 days at 1 and 5 µg/L nominal, respectively, suggesting that steady-state conditions were not achieved. The uptake rate constants (k1) were 48.7 and 14.2 /d and the depuration half-lives were 15 and 17 days for the low and high exposure treatments, respectively. Total elimination rate constants ("k2") were 0.0448 and 0.0407 /d for the low and high exposure treatments, respectively. A growth rate constant ("kG") of 0.021 /d can be calculated from the test data. As the analytical method used did not distinguish between parent compound and metabolites, these values represent the realistic upper limit for the true BCF of the substance and its metabolites. The fish BCF value of 1087 from this study is considered the most reliable. This value is supported by other experimental studies which derived fish BCF values of about 600 for MCCPs tested at around the water solubility, and is also in line with an alternative predictive method (SRC BCF WIN program) which derived a BCF of 1549, as described in the RAR (EU, 2005).There are key technical issues related to data quality, uncertainty and interpretation with study including a lack of correction of mass balance for parent test chemical (i.e., total radioactivity for both parent and possible metabolites was quantified) the position of the radiolabel on the test substance is not reported, and, as previously mentioned, steady-state conditions were not approached during the test (i.e. time to reach ~95% of steady-state = 4xt1/2~60 d). Finally, growth corrected BCF

values, not reported in the original study, of 2164 and 686 were determined for the low and high test concentrations, respectively.

Hurd and Vaughan (2010) is a GLP study conducted according to OECD guideline 305 in rainbow trout using a ^{14}C-labelled n-tetradecane (45% chlorinated) at a single exposure concentration of 0.5 µg/L (nominal) in a flow-through system. The study had 35-day accumulation phase and a 42-day depuration phase. During the accumulation phase, tissue levels of test material, measured by radiochemical analysis, increased to 2265 µg/kg bw (at day 35) at a mean measured exposure concentrations of 0.34 µg/L. During the depuration phase, tissue levels declined to 438 µg/kg bw. The whole body BCF at the end of the exposure phase was 6660 (3230 normalised to 5% lipid content; mean lipid content of the fish was 10.3%). Based on the whole body concentration of test substance equivalents at day 35, the extent of depuration of test substance after 42 days was 81%. Using a kinetic approach, the uptake rate constant (k1) was determined to be 395 /day and the depuration rate constant (k2) 0.0432 /day, giving a kinetic BCF of 9140 (4440 normalised to 5% lipid content). The growth corrected BCF values, not reported in the original study, were determined to be 29924 (fish lipid content =10.3%) and 14526, when normalized for 5% lipid content. The kinetic data also allowed estimates to be made for the depuration half-life (16 days) and the time to 95% of steady state (69 days). Since all these calculations are based on radioactivity measurements, and therefore do not distinguish between parent compound and possible metabolites, these values represent the worst-case values. In conclusion, the whole body BCF for chlorinated n-tetradecane (45% chlorinated) in rainbow trout (Oncorhynchus mykiss) exposed to a nominal concentration of 0.5 µg/l for 35 days was 6660 and the kinetic BCF, based on the calculated uptake and depuration rate constants, was 9140. Normalised to 5% lipid content, these values would be 3230 and 4440, respectively. The BCF of 6660 appears to be unusually high compared with the other BCF values obtained to date, and the method employed may overestimate the BCF with decreasing chlorination content. Samples from this study were subjected to further analysis to determine the proportions of radioactivity present as parent compound or as polar or bound metabolites (Leonards and van Beuzekom, 2010). Only samples of fish at the end of the depuration phase were available. The results showed that no significant extractable metabolites of chlorinated tetradecane were present, but non-extractable tissue metabolites were found, which accounted on average for 21% of the total 14C activity in the fish. It is considered likely that the proportion of metabolites present at the end of the uptake phase would have been different, and potentially higher, than those measured at the end of the depuration phase.

Thompson et al. (2001c) determined the uptake of a radiolabelled C15 chlorinated paraffin (n-pentadecane-8-^{14}C; 51% chlorinated) by earthworms (Eisenia fetida) from soil as part of an earthworm toxicity study. The concentration of MCCPs in adult worms at day 28 was 169, 802 and 732 mg/kg wet wt at measured soil concentrations of 70, 800 and 8200 mg/kg wet wt respectively, resulting in BSAF values of 2.4, 1.0 and 0.089 respectively. For juvenile worms at day 56, the mean tissue concentrations were 140 and 1011 mg/kg wet wt from soil concentrations over 28-56 days of 61 and 748 mg/kg wet wt, resulting in BSAF values of 2.3 and 1.4 (no juvenile worms were produced from adults exposed to the highest soil concentration). In an expert evaluation of the study data (EU, 2005), it was noted that the maximum BCF value in this study of 2.4 was derived from soil containing 4.7% organic carbon. Using a default value of 2% for organic carbon content the maximum BSAF value for MCCPs in earthworms would be estimated to be 5.6. The potential for uptake by worms from soil and sediment appears to reduce with increasing chlorine content of the MCCP. There are some limitations to the use of this study for bioaccumulation testing in the fact that the primary purpose of this study was to evaluate the toxicity of the test material to the earthworm, not bioaccumulation, and the concentrations are much higher than what would have likely been used in a separate B study. Further, it is not recommended to calculate B metrics using total radioactivity, as was done in this study, because it quantifies the parent and

metabolite concentration in the tissues.

Bioaccumulation Factor, Biomagnification Factor and Trophic Magnification Factor Data

There are a range of BAF, BMF and TMF data available on MCCP. These data have been extensively assessed in the reviews by Thompson and Vaughan (2014), published, and Arnot (2014), unpublished (attached in Section 13 of dossier). Both expert assessments concluded that vast majority of these BAF, BMF and TMF data show that MCCP is not bioaccumulating in the food chain, especially when considering samples from the natural environment.

Houde et al. (2008) reported lipid-normalised log BAFs results in the range of 6.5 to 7.3 for MCCP as analyzed based on their field study of various invertebrates and fish in the Great Lakes region of Canada and USA. Lipid-normalised values are generally employed for field data. Houde (2008) also determined a range of field-derived BMFs, which range from 0.11 to 0.94 for total MCCP for various invertebrate to fish in Lake Michigan and Lake Ontario. It should be noted that Houde (2008) also reported a BMF value for sculpin-Diporeia above one in Lake Michigan, though this result was not considered reliable as they are based on a single Diporeia sample as discussed in Houde (2008) and Arnot (2014). For comparison, the sculpin-Diporeia BMF for Lake Ontario was 0.88 for total MCCP in Houde (2008).

The accumulation and depuration of two radiolabelled C16 chlorinated paraffins ([1-^{14}C] n-hexadecane; 34% chlorinated, and [U-^{14}C] n-hexadecane; 69% chlorinated) by oligochaete worms (Lumbriculus variegatus) from spiked sediment has been investigated. Worms were exposed to the 34% chlorinated material at two concentrations (47.1 and 135 µg/g dry weight sediment) and to the 69% chlorinated material at 264 µg/g for 21 days. After transfer to un-spiked sediment, organisms were kept for a further 42 days. Rates of uptake and depuration were determined and kinetic biota-sediment bioaccumulation factors (BSAF) calculated. All determinations were based on measurements of total radioactivity. Recovery of oligochaetes at the end of the study was at least 97%. The rate of uptake of radioactivity from sediment was 0.076 to 0.093 g/g/d for the 34% C16 chlorinated paraffin and 0.013 g/g/d for the 69% material, with equilibrium being reached by 21 days. The BSAF, lipid, organic carbon and growth corrected, was 4.4 for the low chlorination material and 0.6 for the high-chlorination material (Fisk et al. 1998).

The mean bioaccumulation factor (BAF) for a radiolabelled C15 chlorinated paraffin (n-pentadecane-8-14C; 52% chlorinated) from soil by the roots of carrot (Daucus carota) was calculated to be 0.045, based on the relative concentration of radioactivity in soil and carrot root on days 50 to 70 (Thompson et al. 2005).

Overall Bioaccumulation Assessment

Figure 1 is from Arnot 2014 (full report attached in the dossier) and illustrates the application of a bioaccumulation (B) assessment framework proposed in Burkhard et al. (2012). The measured B data for MCCP constituents are from various aquatic species (plankton, invertebrates, fish) from laboratory testing (BCF, BMF) and environmental monitoring (BMF, BAF, TMF). A total of 97 measured data points are compared against the B assessment criterion of 1 (red horizontal line) proposed by Burkhard et al. (2012). Data derived from field studies, and in particular TMF values, are considered to be the ultimate indicator of a compound's potential to bioaccumulate in the natural environment (Gobas 2009). A total of 93% of the data in Figure 1 are from environmental (field) studies and are thus considered highly relevant ("real world") B assessment data. Of these 97 measured data points, 7 (7.2%) met or exceeded the threshold criterion and 93 (92.8%) were lower than the threshold criterion. The median value (central tendency) is 0.27 (black dashed line). The SETAC POP/PBT expert workshop experts

considered that a TMF >1 represented the most conclusive evidence of the bioaccumulative nature of a chemical (Gobas 2009). Figure 1 shows that all of the TMFs for the MCCP constituents < 1. The current weight of evidence indicates that MCCP constituents are not likely to biomagnify in fish and in aquatic food webs.

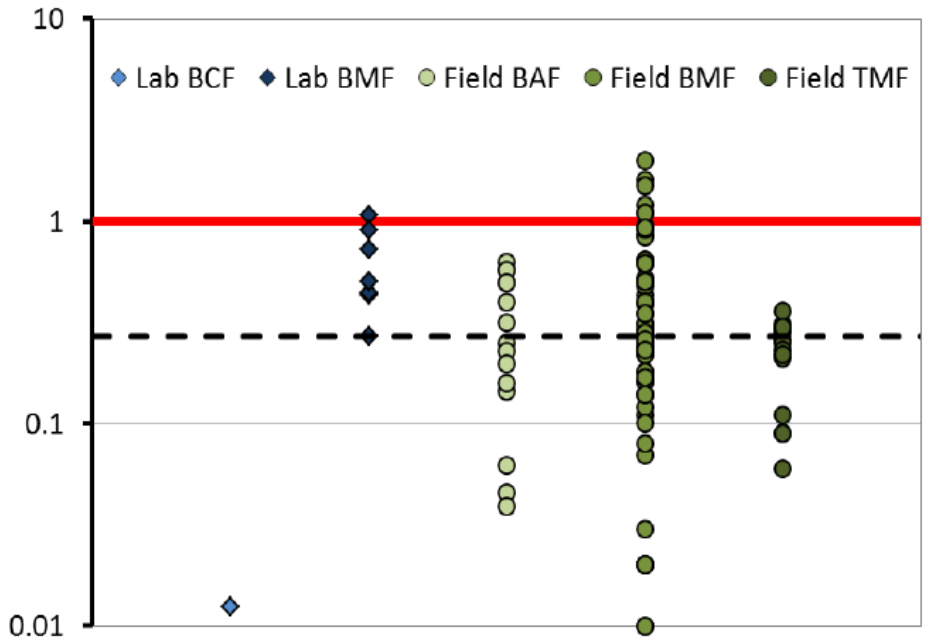


Figure 3. Fugacity ratios calculated using the recommended methods (Burkhard et al., 2012) for available relevant and reliable bioaccumulation data for MCCP constituents. Values > 1 (red line) indicate biomagnification (bioaccumulation) hazard. 93% of the data points are < 1 and the median value = 0.27

CSR References

CG van Ginkel 2010: BIODEGRADABILITY OF CHLORINATED TETRADECANES IN CLOSED BOTTLE TESTS INOCULATED WITH ACTIVATED SLUDGE AND RIVER WATER (study report), Testing laboratory: Akzo Nobel, Report no: 2.427.188. Report date: Dec 1, 2010

CG van Ginkel, 2014: BIODEGRADABILITY OF C15 CHLORINATED N-ALKANE, 51% CL (WT.) IN THE CLOSED BOTTLE TEST (OECD TG 301) (study report), Testing laboratory: Akzo Nobel, Report no: F 14024 CG. Owner company; MCCP REACH Consortium, Report date: Apr 28, 2014

CG van Ginkel 2010: Biodegradability of C14-17 chlorinated paraffin (45.6% Cl) in the closed bottle test (study report), Testing laboratory: AkzoNobel Technology & Engineering, Velperweg 76, 6824 BM Arnhem, The Netherlands, Report no: T10007c. Owner company; Chlorinated paraffins Sector Group, Euro Chlor, 1160 Brussels, Belgium, Report date: Jan 5, 2010

CG van Ginkel 2010: Biodegradability of a C14-17 medium chain chlorinated paraffin (63.2% Cl w/w) in the closed bottle test (study report), Testing laboratory: AkzoNobel Technology & Engineering, Velperweg 76, 6824 BM Arnhem, The Netherlands, Report no: T 10008C. Owner company; Chlorinated Paraffins Sector Group, Euro Chlor, 1160 Brussels, Belgium, Report date: Apr 20, 2010

CG van Ginkel 2018: BIODEGRADABILITY OF C14 CHLORINATED N-ALKANE, 60% Cl

(wt.) IN THE CLOSED BOTTLE TEST (OECD TG 301D) (study report), Testing laboratory: Akzo Nobel, Report no: F17086 CG. Owner company; MCCP REACH Consortium, Report date: Jul 6, 2018

CG van Ginkel 2018: BIODEGRADABILITY OF C14 CHLORINATED N-ALKANE, 55% Cl (wt.) IN THE CLOSED BOTTLE TEST (OECD TG 301D) (study report), Testing laboratory: Akzo Nobel, Report no: F17085 CG. Owner company; MCCP REACH Consortium, Report date: Jul 6, 2018

CG van Ginkel 2010: BIODEGRADABILITY OF MEDIUM-CHAIN CHLORINATED PARAFFIN (51.7% Cl W/W) IN THE CLOSED BOTTLE TEST (study report), Testing laboratory: Akzo Nobel, Report no: 2.424.041. Owner company; MCCP REACH Consortium, Report date: Dec 1, 2010

CG van Ginkel 2010: Biodegradability of polychlorinated tetradecane (45%) in the closed bottle test (study report), Testing laboratory: AkzoNobel Technology & Engineering, Velperweg 76, 6824 BM Arnhem, The Netherlands, Report no: 2.397.140. Owner company; Chlorinated Paraffins Sector Group, Euro Chlor, B-1160 Brussels, Belgium, Study number: T09050 C, Report date: Jan 5, 2010

CG van Ginkel 2018: BIODEGRADABILITY OF C14 CHLORINATED N-ALKANE, 50% Cl (wt.) IN THE CLOSED BOTTLE TEST (OECD TG 301D) (study report), Testing laboratory: Akzo Nobel, Report no: F17084 CG. Owner company; MCCP REACH Consortium, Report date: Jul 6, 2018

CG van Ginkel 2018: BIODEGRADABILITY OF C15 CHLORINATED N-ALKANE, 51% Cl (wt.) IN THE CLOSED BOTTLE TEST (OECD TG 301D) (study report), Testing laboratory: Akzo Nobel, Report no: F17032 CG. Owner company; MCCP REACH Consortium, Report date: Jul 6, 2018

van Ginkel, C.G. 2014: BIODEGRADABILITY OF C15 CHLORINATED N-ALKANE, 51% CL (WT.) IN THE CLOSED BOTTLE TEST (OECD TG 301D) (study report), Testing laboratory: Akzo Nobel, Report no: F 14025 CG. Owner company; MCCP REACH Consortium, Report date: Apr 28, 2014

Madeley JR and Birtley RDN 1980: Chlorinated paraffins and the environment. 2. Aquatic and avian toxicology (publication), Environ Sci Technol. 14: 1215-1221. Report date:

EU 2005: Alkanes, C14-17, chloro (MCCP). Part 1 Environment (publication), European Union Risk Assessment Report. Report no: Vol 58. Report date:

CG van Ginkel 2010: EVALUATION OF THE ULTIMATE BIODEGRADABILITY OF CHLORINATED TETRADECANES USING IN SEQUENCING BATCH REACTORS (study report), Testing laboratory: Akzo Nobel, Report no: 2.427.189. Report date: Dec 1, 2010

J. Coleman and E. Schaefer 2019: C14 CHLORINATED PARAFFIN: AEROBIC TRANSFORMATION IN AQUATIC SEDIMENT SYSTEMS (study report), Testing laboratory: EAG Easton, Owner company; MCCP REACH Consortium, Study number: 835E-104, Report date: Apr 26, 2019

Fisk AT, Wiens SC, Webster GRB, Bergman A and Muir DCG 1998: Accumulation and depuration of sediment-sorbed C12- and C16-polychlorinated alkanes by oligochaetes (Lumbriculus variegatus) (publication), Envir. Toxicol. Chem 17: 2019-2026.. Report date:

Omori T, Kimura T and Kodama T 1987: Bacterial cometabolic degradation of chlorinated paraffins (publication), Appl. Microbiol. Biotechnol. 25:553-557.. Report date:

Hurd KS and Vaughan M 2010: [14C]-chlorinated n-tetradecane: determination of the bioconcentration in Rainbow trout (Oncorhynchus mykiss) (study report), Testing laboratory: Brixham Environmental Laboratory, Brixham, Devon TQ5 8BA, UK, Report no: BR0088/B. Owner company; Chlorinated Paraffins Sector Group, Euro Chlor, Avenue E. Van Nieuwenhuyse 4, Box 2, B-1160 Brussels, Belgium, Report date: Mar 16, 2010

2018: C14 CHLORINATED PARAFFIN: A DIETARY EXPOSURE BIOACCUMULATION TEST WITH THE RAINBOW TROUT (Oncorhynchus mykiss) (study report), Testing laboratory: Eurofins EAG (Easton); VU (Amsterdam), Owner company; MCCP REACH Consortium, Study number: 835A-101, Report date:

Thompson RS, Caunter JE and Gillings E 2000: Medium-chain chlorinated paraffin (51% chlorinated n-pentadecane-8-14C): Bioconcentration and elimination by rainbow trout (Oncorhyncus mykiss) (study report), Testing laboratory: AstraZeneca, Brixham, Devon, TQ5 8BA, Report no: BL6869/B. Owner company; Euro Chlor, Brussels, Belgium, Report date: Jun 5, 2000

Aaron T. Fisk, Chris D. Cymbalisty, Gregg T. Tomy, Derek C.G. Muir 1998: Dietary accumulation and depuration of individual C10-, C11- and C14-polychlorinated alkanes by juvenile rainbow trout (Oncorhynchus mykiss) (publication), Aquatic Toxicology 43 (1998) 209-221. Report date:

AARON T. FISK, GREGG T. TOMY, CHRIS D. CYMBALISTY, and DEREK C.G. MUIR 2000: DIETARY ACCUMULATION AND QUANTITATIVE STRUCTURE-ACTIVITY RELATIONSHIPS FOR DEPURATION AND BIOTRANSFORMATION OF SHORT (C10), MEDIUM (C14), AND LONG (C18) CARBON-CHAIN POLYCHLORINATED ALKANES BY JUVENILE RAINBOW TROUT (ONCORHYNCHUS MYKISS) Environmental Toxicology and Chemistry, Vol. 19, No. 6, pp. 1508–1516, 2000. Report date:

H.M. Cooley, A.T. Fisk, S.C. Wiens, G.T. Tomy, R.E. Evans, D.C.G. Muir 2001: Examination of the behavior and liver and thyroid histology of juvenile rainbow trout (Oncorhynchus mykiss) exposed to high dietary concentrations of C10-, C11-, C12- and C14-polychlorinated n-alkanes (publication), Aquatic Toxicology 54 (2001) 81–99. Report date:

Thompson RS, Smyth DV and Gillings E 2005: Medium-chain chlorinated paraffin (C14-17, 52% chorinated) : Accumulation from soil by the roots of carrot (Daucus carota) (study report), Testing laboratory: Brixham Environmental Laboratory, Brixham, Devon TQ5 8BA, Report no: BL8221/B. Report date: Dec 21, 2005

Arnot JA, Mackay D, Bonnell M. 2008. Estimating metabolic biotransformation rates in fish from laboratory data.Environ Toxicol Chem27:341-351.

Arnot, J. 2014. Bioaccumulation Assessment of Medium-chain chlorinated paraffins (MCCPs). April 28, 2014. Unpublished reported provided in Section 13 of the REACH dossier. ARC Arnot Research & Consulting Inc., Toronto, ON.

Arnot J. 2013. Comments on Preliminary Bioaccumulation Assessment of Medium Chain Chlorinated Paraffins (MCCPs). ARC Arnot Research & Consulting Inc., Toronto, ON.

Burkhard LP, Arnot JA, Embry MR, Farley KJ, Hoke RA, Kitano M, Leslie HA, Lotufo GR, Parkerton TF, Sappington KG, Tomy GT, Woodburn KB. 2012. Comparing laboratory and field measured bioaccumulation endpoints.Integr Environ Assess Manage8:17–31.

Connolly JP, Pedersen CG. 1988. A Thermodynamic-Based Evaluation of Organic Chemical Accumulation in Aquatic Organisms. Environ Sci Technol 22:99-103.

EAG. 2018: C14 CHLORINATED PARAFFIN: A DIETARY EXPOSURE BIOACCUMULATION TEST WITH THE RAINBOW TROUT (Oncorhynchus mykiss) (study report), Testing laboratory: Eurofins EAG (Easton); VU (Amsterdam), Owner company; MCCP REACH Consortium, Study number: 835A-101 Alkanes, C14-17, chloro (updated May 2019)

Gobas, FAPC, de Wolf W, Burkhard L, Verbruggen E, Plotzke K. (2009) Revisiting Bioaccumulation Criteria for POPs and PBT Assessments.Integr Environ Assess Manage5(4): 624–637.

Leonards, Pim, van Mourik, Louise. 2019. 1-Octanol/water partition coefficient determination of C14 polychlorinated n-alkane with 50% Cl by weight. VU Amsterdam. March 18, 2019.

Leonards, Pim, van Mourik, Louise. 2019. Water solubility determination of C14 polychlorinated n-alkane with 50%Cl by weight. VU Amsterdam. 14 August 2019.

Mackay D. 1982. Correlation of Bioconcentration Factors.Environ Sci Technol16:274-278. Nichols JW, Fitzsimmons PN, Burkhard LP. 2007.In vitro-in vivo extrapolation of quantitative hepatic biotransformation data for fish. II. Modeled effects on chemical bioaccumulation.Environ Toxicol Chem26:1304-1319.

OECD. 2012. OECD Guidelines for Testing Chemicals. Test No. 305: Bioaccumulation in Fish: Aqueous and Dietary Exposure. Organization for Economic Co-operation and Development, Paris.

Thompson R, Vaughn M. 2014. Medium-chain chlorinated paraffins (MCCPs): A review of bioaccumulation potential in the aquatic environment. Integr Environ Assess Manage10(1): 78–86.