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IN VIVO COMPARISON OF THE PROANGIOGENIC PROPERTIES OF CHLORDECONE AND THREE OF ITS DECHLORINATED DERIVATIVES FORMED BY IN SITU CHEMICAL REDUCTION

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- 30 KEYWORDS: In situ chemical reduction, chlordecone, dechlorination, angiogenesis, prostate cancer
- 31
- 32

33 ABSTRACT

34 In Situ Chemical Reduction has been identified as a possible way for the remediation of soils contaminated by chlordecone (CLD). Evidences provided by the literature indicate an 35 36 association between the development of prostate cancer and CLD exposure (Multigner et al. 37 2010). In a previous study, we demonstrated that the two main CLD derivatives obtained by 38 the reduction method: CLD-1Cl and CLD-3Cl have lower cytotoxicity and proangiogenic 39 properties than CLD itself (Legeay et al. 2017). By contrast, nothing is known on the 40 proangiogenic effect *in vivo* of these dechlorinated products. The aim of this study is to confirm the preliminary in vitro results on an in vivo model of human prostate cancer. 41

42 The proangiogenic effect of dechlorinated derivatives of chlordecone was evaluated on a 43 murine model of human prostate tumor (PC-3) treated with CLD, CLD-1Cl, CLD-3Cl and 44 CLD-4Cl at two exposure levels: 33 μ g/kg and 1.7 μ g/kg respectively reflecting acute and 45 chronic toxic exposure in human (Adir et al. 1978); (Cohn et al. 1978).

46 The results of the serum concentration measurements shown that, for the same ingested dose,

these three metabolites were significantly less present in the serum than CLD, meaning that
dechlorination of CLD lead to molecules that are biologically faster absorbed or metabolized
or both.

50 Evolution of prostate tumors growth was lower in the groups treated by the three metabolites 51 compared to the one treated by CLD. The vascularization measured on the tumor sections was 52 inversely proportional to the rate of dechlorination, the treatment with CLD-4Cl showing no

53 difference with control animals treated with the vehicle oil used for all substances tested.

54 We can therefore conclude that pro-angiogenic effect of the CLD is significantly decreased

55 following the ISCR-resulting dechlorination, and that the carcinogenic effect is reduced.

56 Further investigations are now needed to elucidate the molecular mechanisms by which

57 dechlorination of CLD reduces its carcinogenic and proangiogenic effects in prostate tumor.

59 **INTRODUCTION**

60 Chlordecone (CLD, Kepone, decachlorooctahydro-1,3,4-metheno-2Ha.k.a. 61 cyclobuta[c,d]pentalen-2-one, CAS No: 143-50-0) is a chlorinated hydrocarbon insecticide, 62 now classified as a persistent organic pollutant (POP). CLD has been intensively used in banana plantations of the French West Indies, as well as, to a lesser extent, in other countries (POPRC 63 64 2007; Le Déault and Procaccia 2009; Joly 2010; Fintz 2010). This pesticide undergoes no significant biotic or abiotic degradation in the environment (Cabidoche et al. 2009), leading to 65 chronic food contamination and accumulation in liver, muscles, and fat of animals (Clostre et 66 67 al. 2014; Létondor et al. 2015; Lastel et al. 2016). These effects explain its high toxicity that lead to its global prohibition (Cannon et al. 1978; Landrigan et al. 1980). CLD pollution is 68 69 bound to last for centuries if nothing is done to lower soil contamination (Cabidoche et al. 70 2009). Such persistence in the environment is a major health problem. Indeed, several studies 71 have previously reported that chronic exposure to CLD leads to hepatotoxicity and increases 72 the risk of cancer in rats (Reuber 1979; Mehendale 1981; Guzelian 1982; Dalu and Mehendale 73 1996; Murali et al. 2004). In Human, it significantly increases the risk of prostate and liver 74 cancers, and pregnancy complications (Reuber 1979; Sirica et al. 1989; Faroon et al. 1995; 75 Multigner et al. 2010; Dallaire et al. 2012; Kadhel et al. 2014; Multigner et al. 2016). 76 Furthermore, previous study has reported that CLD is able to enhance *in vitro* angiogenesis 77 through the activation of estrogen receptors, thus favoring both tumor growth and development 78 (Clere et al. 2012).

79 In situ chemical reduction (ISCR) may be a solution for the remediation of soils contaminated 80 by CLD (Clostre et al. 2010). ISCR (United States Patent 2000) is based on treatment cycles of alternated strongly reducing conditions with oxidizing conditions and has already been proven 81 82 effective in remediation of soils contaminated by pesticides (Phillips et al. 2004, 2005, 2006; 83 Kim et al. 2010). Among all the metabolites, only the major transformation products, *i.e.* a 84 mono dechlorinated CLD: chlordecone - 1Cl (CLD-1Cl) and chlordecone - 3Cl (CLD-3Cl) 85 were commercially available and tested. The CLD-1Cl and CLD-3Cl (the data available do not 86 enable the position of dechlorination to be more precisely defined (Belghit et al. 2015)) 87 represents 50 % and 13% respectively of the cumulated relative peak areas of the eleven 88 transformation products identified after the 27-day ISCR treatment of an alluvial soil 89 concerning the hypothetic malignant effects of these metabolites (Mouvet et al.). A third

- 90 derivative even more dechlorinated, quadri-hydro CLD (CLD-4Cl) is also observed, with a
- 91 much lower relative area than CLD-3Cl.
- 92 Recently, we have demonstrated from *in vitro* data, that CLD-1Cl and CLD-3Cl have lower
- 93 proangiogenic properties compared to CLD (Legeay et al. 2017). While the *in vivo* effects of
- 94 CLD-1Cl and CLD-3Cl and their properties on tumor growth remain unknown, the aims of this
- 95 study were to evaluate the influence of CLD and its metabolites in the control of
- 96 neovascularization in a mice model of prostate cancer.

97

99 MATERIALS AND METHODS

100 *Reagents and chemicals*

101 CLD, purity 99.5%, was purchased from Dr. Ehrenstorfer (reference n12291). CLD-1Cl, CLD102 3Cl and CLD-4Cl formed by ISCR were synthesized by Alpha-Chimica (Alpha-Chimica,
103 Châtenay-Malabry, FRANCE) with a purity of 97.2%, 97.4% and 95.8%, respectively. GC104 MS/MS shows the impurities to be other CLD dechlorinated derivatives. PC-3 cells (CRL105 1435) were obtained from ATCC (Manassas, VA). RPMI 1640 medium, F-12 nutrient mix,
106 penicillin-streptomycin, fetal bovine serum (FBS), were purchased from Invitrogen (Carlsbad,

- 107 CA). Anti-CD31 antibody was purchased from (BD Biosciences). Biotinylated second antibody
- 108 IgG was purchased from (Vector BA-9401). ECM gel[®] was purchased from (Sigma-Aldrich).
- 109 CYQUANT[®] direct cell proliferation kit was purchased from (Invitrogen[®]).
- 110

111 *Cell culture*

Human umbilical venous endothelial cells (HUVEC) obtained from male newborns were purchased from Lonza (CC2519) and grown in plastic flasks in EBM-2 medium (Lonza, CC3156), containing 1% streptomycin/penicillin (Lonza, DE17-602E) and 10% of heatinactivated fetal bovine serum (FBS, Gibco, 10270-106). HUVEC were used between the second and fourth passage.

Prostate cancer cell line PC-3 were purchased from ATCC (CRL-1435) and grown in plastic flasks in complete RPMI 1640 medium (ThermoFisher, 11875-085), containing 2 mM of Lglutamine, 20 mM of HEPES, 1% streptomycin/penicillin (Lonza, DE17-602E) and 10% of heat-inactivated fetal bovine serum (FBS, Gibco, 10270-106). PC-3 were used between the first and fifth passage.

122

123 Chlordecone and metabolite dose calculations for in vivo experiments

124 Important points were considered when calculating the drug concentrations/doses for *in vivo* 125 studies: i) dose calculations for oral administration of CLD and its metabolites *in vivo*, as 126 compared with intravenous administration in mice, and ii) comparison of exposure to the 127 molecules in mice serum with that observed in humans serum.

i) As bioavailability is about 90% in the rat (Egle et al. 1978), and the most common way of
intoxication is the ingestion, the oral administration route has been chosen to administrate the
molecules.

- 131 ii) Two targeted serum concentrations have been selected: 500 μ g/L as the reflect of high
- 132 exposure in humans (Adir et al. 1978), and 25 μ g/L as the exposure of industrial workers (Cohn
- 133 et al. 1978). As mice weights 29.87 \pm 0.39 g and have about 2 mL of blood, doses of 33 μ g/kg
- 134 and 1.7 μ g/kg have been chosen.

135 Xenograft model

- 136 PC-3 cell suspension (1.5.10⁶ cells in 150 μ L PBS w/o FBS) was xenografted subcutaneously
- 137 into the right flank of 5-weeks-old male *nude* mice (Envigo, Venray, N.L.). All animal138 procedures listed in this article were performed as per the protocol approved by the local ethic
- 139 committees (Angers, France; protocol APAFIS #5872) and the number of animals was limited
- 140 regarding animal ethics.
- 141 The 41 mice were then divided randomly into nine groups. Eight groups were exposed to CLD

142 or its derivatives respectively at 33 μ g/kg and at 1.7 μ g/kg of body weight by daily gavage in

143 200 µL alimentary virgin olive oil for 8 weeks. The control group received daily gavage with

- 144 200 µL alimentary virgin olive oil for 8 weeks. The experimental plan followed is described in
- 145 figure 1.
- 146



147

Figure 1: Distribution of animals according to the conditions of exposure to different compounds.

- 150
- 151 Tumor volume has been measured each 2 days according to the formula:
- 152 Tumor volume = $[1/2(\text{length x width}^2)]$ (Euhus et al. 1986).
- 153 Mice were sacrificed after 8 weeks and tumors were measured, dissected, weighed, and snap
- 154 frozen in Tissue-Tek[®] O.C.T. compound and stored at -80°C for further analysis.

155

156 Serum concentrations measurement

Whole blood was collected into a dry polypropylene microtube by direct intracardiac puncture
under anesthesia 24 h after the last intake. Samples were left at room temperature for 30 min
until blood clotting (Tuck et al. 2009). The clot was then removed by centrifugation at 1,000 *g*for 10 min at room temperature, and the supernatant has been stored at -80°C until analysis.
The molecules of interest: CLD, CLD-1Cl, CLD-3Cl, CLD-4Cl but also chlordecol (CLDOH)
and 5b-hydro-chlordecone (5bCLD) were then measured in serum samples as previously

described (Bichon et al. 2015). Briefly, internal standard (labeled CLD: 13C10-CLD at 10 163 164 $pg/\mu L$) was added to 50 μL of mouse serum. Fat and proteins were then precipitated with 50 μl 165 formic acid by vortexing for 10 sec. Supernatants were loaded onto a C18 SPE cartridge, 166 washed with hexane, eluted with acetonitrile and dried under a nitrogen stream. Dried extracts 167 were reconstituted in 25 µL of a water/acetonitrile mix and injected into the LC-ESI-MS/MS 168 instrument (Acquity-XevoTOS, Waters). Molecules were separated using an Accucor C30 169 column (Thermo) with a water/acetonitrile gradient. Targeted compounds were analyzed in 170 SRM mode, except for chlordecol in SIM mode. The calibration range was from 0.5 to 50 pg/µL 171 of serum, and the limits of detection and quantification (LOQ) were from 0.02 to 3.83 pg/µL

depending on the compound. Average recoveries into serum were between 63 to 91%.

173

174 Immunochemistry and vessel area quantification

Tumor sections (7 μ m) were fixed with paraformaldehyde 10 min at 4°C, then, blocked with 4% bovine serum albumin for 1 h at room temperature and incubated at 4°C with the primary anti-CD31 antibody (10 μ g/mL, Purified Rat Anti-Mouse CD31, BD Biosciences) at 1:500 dilution. Then, tissues were incubated with a biotinylated second antibody IgG (Biotinylated Goat Anti-Rat IgG Antibody, mouse adsorbed, Vector BA-9401) at 1:200 dilution for 60min at room temperature and counterstained with DAPI (4',6-diamidino-2-phenylindole) at 1:10,000 dilution for 3 min.

182 Vessels were quantified in the most representative areas, as identified by scanning tumor 183 sections at low power (x40) with a confocal microscope. The total areas occupied by tumor 184 vessels identified by CD31 staining in 3 spots per tumor, were calculated with ImageJ software.

185

186 In vitro capillary network formation on ECM gel®

- 187 HUVECs were seeded with a density of 10^4 cells per well previously covered with ECM gel[®]
- 188 (Sigma-Aldrich). Briefly, $10 \,\mu L \text{ of ECM}^{\$}$ was added into a fifteen-well plate Ibidi^{\$} and allowed
- to solidify for 45 min at 37°C. Cells were then incubated with medium containing 10% FBS in
- 190 presence of CLD or CLD-4Cl at concentrations 5.10⁻⁸ M, 10⁻⁹ M or 10⁻¹¹ M and allowed to
- adhere for 24 h. Tube formation was examined by phase-contrast microscopy (MOTIC AE21)
- after 24 h and average length of capillaries was quantified using ImageJ software for evaluation
- 193 of angiogenesis.
- 194

195 Proliferation test on tumor cells PC-3

The effect of CLD, CLD-1Cl, CLD-3 and CLD-4Cl on the proliferation of human prostate cells 196 PC-3 (ATCC[®], CRL-1435) was analyzed using CYQUANT[®] assay kit (Invitrogen[®]). 5.10³ 197 cells were seeded into a 96-well plate for 24 h and then were treated with 10⁻⁶M and 5.10⁻⁸M 198 199 CLD, CLD-1Cl, CLD-3 and CLD-4Cl at concentrations (these reflect the target serum 200 concentrations at 33µg/kg and 1.7µg/kg respectively) for 24h. 100µL of detection reagent mixture was then added (Cyquant[®] direct nucleic acid stain and Cyquant[®] direct background 201 202 suppressor 1) to 100µL of culture medium per well and the cells were incubated at 37°C for 60 203 min. Fluorescence levels were read by a fluorescent microplate reader (Clariostar) with a filter 204 at 508 nm for excitation and 527 nm for emission.

205

206 Statistical analysis

The evolution of tumor size, different concentrations in the serum and area occupied by the neo-vessels were compared between the different groups with ANOVA t-test. p<0.05 was considered statistically significant.

211 **RESULTS**

- Evolution of tumor growth under chlordecone or its dechlorinated derivatives exposure
 in mice.
- The objective of this analysis is to compare the evolution of tumor growth in mice treated with
- 215 CLD or its derivatives.
- 216 In low dose groups ($1.7\mu g/kg$), a significant increase of tumor volume was found in mice treated
- with CLD and CLD-1Cl compared with controls (p=0.028 and p=0.035 respectively) (**Fig 2A**).
- 218 No significant difference of tumor growth was observed in mice treated with CLD-3Cl and
- 219 CLD-4Cl compared to control mice.
- 220 In high dose groups (33 μ g/kg), a significant increase of tumor growth has been observed in
- 221 mice treated with CLD in comparison with control animals (p=0.01). No significant difference
- 222 was observed between mice treated with CLD and mice treated with CLD-1Cl while tumor
- 223 growth was significantly lower for mice treated with CLD-3Cl and CLD-4Cl compared to mice
- treated with CLD (p=0.035 and p=0.025 respectively) (Fig 2B). In addition, no significant
- 225 difference was observed between CLD-3Cl-treated mice and CLD-4Cl-treated mice compared
- to control mice.



228

Figure 2: Daily treatments for 8 weeks at low (1.7 μg/kg) and high (33 μg/kg) dose with
 CLD but not with CLD-3Cl and CLD-4Cl increase tumor growth formed by PC-3 cells
 in mice compared to control mice. *p<0.05; **p<0.01. n=3-12.

232

233 2. Serum dosage of chlordecone and its dechlorinated derivatives

After the low dose exposure of CLD (1.7 μ g/kg), the mean serum concentration reached 26.3 µg/L and the mean serum concentration after high dose exposure of CLD (33 µg/kg) reached 192.8 µg/L. However, the same dose of dechlorinated CLD derivatives lead to very lower serum concentrations with the following order: CLD > CLD-1Cl > CLD-3Cl > CLD-4Cl and values ranging from 67.1±21.2 µg/L to under the limit of detection respectively for 33 µg/kg of CLD-1Cl and 1.7 µg/kg of CLD-4Cl.

- 240 These serum dosages also reveal that for a same dose, chronicle ingestion of all dechlorinated
- $\label{eq:cld} 242 \qquad \text{dechlorination: } CLD > CLD-1Cl > CLD-3Cl > CLD-4Cl.$
- 243 It is interesting to note that the treatment with a low dose of CLD-4Cl leads to a serum
- concentration lower than the limit of detection. In addition, the presence of impurities like CLD,
- 245 CLD-1Cl and CLD-3Cl have also been found but at concentrations 5 to 175 times lower than
- the treatment in serum of mice (data not shown).
- 247

	Concentration, mean \pm SD (µg/L)			
	CLD	CLD-1Cl	CLD-3Cl	CLD-4Cl
CLD	263+127			
1.7µg/kg	20.3 ± 12.7			
CLD	$192.8 \pm$			
33µg/kg	73.6			
CLD-1Cl		11.9 ±3.2		
1.7µg/kg				
CLD-1Cl	67.1 ±	67.1 ± 21.2		
33µg/kg		07.1 ± 21.2		
CLD-3Cl		4.0 + 2.5		
1.7µg/kg			4.0 ± 5.5	
CLD-3Cl			61 ± 43	
33µg/kg		0.1 ± 4.5		
CLD-4Cl				<100
1.7µg/kg				< LUQ
CLD-4Cl				22 + 0.9
33µg/kg				2.2 ± 0.9

Table 1: Concentrations of CLD and its derivatives measured in mice serum samples.

- 250 3. Effect of chlordecone and its dechlorinated derivatives on the proliferation of PC-3 tumor
- 251 cells
- 252 No difference was found in PC-3 cell proliferation whatever the concentration and the type of
- 253 dechlorinated CLD derivative (**Fig 3**).
- 254



Figure 3: Chlordecone or its derivatives even after 72h of exposure do not increase the proliferation of PC-3 tumor cells. n=6.

258

255

4. Effect of chlordecone and its dechlorinated derivatives on *in vivo* neovascularization Treatments of mice with each dose of CLD significantly increased capillary area in tumor compared to control condition (p=0.0007 for 1.7 μ g/kg and p=0.0009 for 33 μ g/kg) (**Fig 4**). Furthermore, capillary areas were significantly reduced in tumors from mice treated with the dechlorinated CLD derivatives (CLD vs CLD-1Cl, p=0.01 and p=0031; CLD vs CLD-3Cl, p=0.0003 and p=0.0005; CLD vs CLD-4Cl, p=0.0007 and p=0.0009 respectively for the low and the high dose) (Fig 3).







High dose 33µg/kg



267

Figure 4: Dechlorination of CLD decreases the tumor neovascularization. A)
Immunolabling of tumor sections. In blue the labeling of cell nucleus with DAPI and in

270green the labeling the endothelial marker CD31. (Scale 50 μm). B and C) Quantification271of the neovascularization. *p<0.05, **p<0.01, ***p<0.001. n=6</td>

272

273

5. Effect of chlordecone and its dechlorinated derivatives on *in vitro* angiogenesis

274 In vitro assays performed in a previous study suggested that CLD-1Cl and CLD-3Cl have lower 275 proangiogenic properties than the CLD parent molecule on human primary endothelial cells 276 (Legeay et al. 2017). Because the present study has an additional derivative, CLD-4Cl, 277 compared to the above-mentioned work, the opportunity has been taken to complete in vitro data by treating HUVECs in the presence or absence of CLD or CLD-4Cl at the same three 278 concentrations 5.10⁻⁸ M (concentration that has been found in plasma of patients exposed), 10⁻ 279 ¹¹ M (concentration that has been found in drinking water) and 10⁻⁹ M (intermediate 280 concentration) for 24h. It has been found that CLD-4Cl does not significantly increase capillary 281 formation versus control cells for the three concentrations tested (5.10⁻⁸ M, p = 0.064; 10⁻⁹ M, 282 p = 0.063; 10⁻¹¹ M, p = 0.1). Additionally, it has been observed that CLD-4Cl possesses a 283 significantly lower proangiogenic effect than CLD (5.10^{-8} M, p = 0.004; 10^{-9} M, p = 0.003; 10^{-9} 284 ¹¹ M, p = 0.009) (Fig 5 and Table 2). 285



296 **p<0.01 vs CLD n=6

300 **DISCUSSION**

301 In order to confirm our previous in vitro data (Legeay et al. 2017), this study aimed to evaluate 302 the proangiogenic properties of dechlorinated derivatives of CLD compared to CLD in a mouse 303 model of tumor-xenograft and *in vitro*. In line with the literature, CLD potentiates prostatic 304 tumor growth in vivo (Multigner et al. 2010) and this effect was associated with an increased 305 neovascularization (Clere et al. 2012; Legeay et al. 2017; Alabed Alibrahim et al. 2018), 306 confirming the proangiogenic effects of CLD. Prostate cancer is one of the most common 307 cancer diagnosed in men and higher incidence rates have been observed in regions highly 308 exposed to CLD like Caribbean (Center et al. 2012). A causal relationship between CLD exposure and prostate cancer has been well described (Multigner et al. 2010; Nedellec et al. 309 310 2016a, b; Deloumeaux et al. 2017) and any possible way of lowering permanently the CLD soil 311 concentrations and the exposure levels of Caribbean inhabitants remains of major interest.

Decontamination of soils by ISCR leads to dechlorinated CLD derivatives which have been previously identified with less or absence of proangiogenic effects (Legeay et al. 2017). From *in vitro* and *in vivo* models, the present study confirms that the proangiogenic properties of CLD derivatives decrease according to the level of dechlorination (Fig 6).

316



Figure 6: The levels of dechlorination leads to less or absence of pro-angiogenic effects
compared to CLD. Combined data from the present study and Legeay S. *et al* (2017). *p<0.05;
p<0.01; *p>0.001. n=6.

321

317

These results have been associated with both the absence of PC-3 cell proliferation and a decreased tumor vascularization in mice treated with CLD-3Cl and CLD-4Cl compared to CLD. These effects were also associated with a decreased capillary formation by human primary endothelial cells.

The present study has been designed to obtain an *in vivo* murine model reflecting individual prostate cancer patients. Thus, mice bearing PC-3 cells have been daily treated by gavage for 8

weeks with two doses: 1.7 μ g/kg and 33 μ g/kg in order to reach 25 μ g/L (5.10⁻⁸ M), the serum 328 329 concentration in industrial workers, and 500 μ g/L (10⁻⁶ M), the serum concentration after high 330 human exposure respectively described with CLD (Adir et al. 1978; Cohn et al. 1978). The 331 serum concentrations of CLD measured after 8 weeks was 26.7±12.7 µg/L which is very close 332 to the targeted serum concentration and confirms our extrapolations for the pharmacokinetics 333 of CLD in mice. However, for the highest dose 33 µg/kg, the serum concentration of CLD was 334 192.8 \pm 7.6 µg/L while the targeted serum concentration was 500 µg/L. This decrease of the 335 observed serum concentration would be partly explained by a possible bioaccumulation in 336 organs such as liver, fat or kidney, leading to a lower CLD concentration in the blood 337 compartment as previously described after oral exposure to CLD (Cohn et al. 1978; Rochelle 338 and Curtis 1994; Faroon et al. 1995). However, the same dose of dechlorinated CLD derivatives 339 leads to very lower serum concentrations with the following order: CLD > CLD-1Cl > CLD-340 3Cl > CLD-4Cl and values ranging from $67.1\pm21.2 \ \mu g/L$ to under the limit of detection 341 respectively for 33 µg/kg of CLD-1Cl and 1.7 µg/kg of CLD-4Cl. These data suggest a decrease 342 of the oral bioavailability or an increase of the metabolism or both of the CLD derivatives 343 compared to CLD. This hypothesis should be verified by deeper pharmacokinetic studies. The 344 presence of CLD and some of its derivatives at very low concentration in serum of treated mice 345 might be due to the presence of impurities in the original product and/or be explained by cross-346 contaminations. However, because their concentrations were 5 to 175 times lower, they can be 347 considered as negligible.

348 According to the two targeted serum concentrations 25 μ g/L (5.10⁻⁸ M) and 500 μ g/L (10⁻⁶ M),

349 PC-3 cell proliferation has been evaluated after exposure to CLD and its derivatives with these 350 both concentrations for 24h to 72h. In addition, the evaluation of the angiogenic process has 351 been performed in human primary endothelial cell after exposure to CLD or its derivatives for 24h at concentrations from 10⁻¹¹ M to 5.10⁻⁸ M, in order to reflect both the lowest serum 352 353 concentration described in exposed human and the CLD concentration found in drinking water 354 (Adir et al. 1978; Landrigan et al. 1980; Taylor 1982; Badach et al. 2000) according to our 355 previous study (Legeay et al. 2017). Altogether, these *in vitro* data confirm that even at very 356 low concentration, CLD and CLD-1Cl but not CLD-3Cl nether CLD-4Cl have proangiogenic 357 effects while no proliferation of PC-3 cell are observed, certifying that the *in vivo* tumor growth 358 after chronicle exposure is at least due to a stimulation of angiogenesis process.

359 It is interesting to note that the lower proangiogenic and tumorigenic properties of dechlorinated
360 derivatives compared to CLD are correlated with the level of chlorination. From *in vivo* data,
361 CLD effectively exhibits the most potentiation of tumor growth while CLD-3Cl and CLD-4Cl

have limited effect presenting no significant differences compared to control mice. This decrease of toxicity is also observed with the neovascularization immune staining of tumor sections and *in vitro* with de capillary length formed by primary endothelial cells. These *in vitro* data confirm that the decreased of the proangiogenic effect following the dechlorination of the CLD derivatives is well due to the dechlorination itself and not to a decrease of bioavailability or an increased metabolism suggested *in vivo*.

368 While no study has evaluated the impact of structural modifications of toxic molecules on the 369 regulation of angiogenesis, a decrease of toxicity such as cell proliferation, cellular functions 370 and mammalian cells alterations and death have been previously observed with polychlorinated 371 biphenyls (Ganey and Boyd 2005; Beyer and Biziuk 2009; Gui et al. 2013) and CLD (Dolfing 372 et al. 2012; Benoit et al. 2017). Interestingly, these cellular damages were correlated with the 373 level of reductive dechlorination, suggesting that more the compound is dechlorinated and less 374 its toxicity is. In endothelial cells, a reduced inflammation through VCAM, COX-2 and NF- κ B 375 expression and a reduced oxidative stress have been observed with biphenvl, the final 376 dechlorination product of PCB77 (Venkatachalam et al. 2008; Eske et al. 2014). In these 377 studies, it was clearly demonstrated that the cytotoxicity was decreased relatively to the degree 378 of dechlorination. Furthermore, the dechlorination of highly chlorinated compounds was 379 suggested to be beneficial for protecting vessels against vascular diseases (Venkatachalam et 380 al. 2008; Eske et al. 2014).

381 A lot of research has been focused on process inducing reductive dechlorination of 382 polychlorinated pollutants in order to increase their biodegradation and consequently to 383 decrease their environmental toxicity. Bioremediation using microorganisms, plants or 384 enzymes, has been wildly employed to detoxify the soil from chlorinated contaminants (Wiegel 385 and Wu 2000; Borja et al. 2005; Jesus et al. 2016). However, these process did not last more 386 than 6 months for CLD (Woignier et al. 2012, 2013). ISCR has already demonstrated its 387 efficiency for the remediation of polychlorinated compounds as hexachlorocyclohexane (HCH) 388 (Phillips et al. 2004, 2005, 2006; Kim et al. 2010) and was suggested as a future way to solve 389 the soil pollution with CLD (Clostre et al. 2010).

- 390 Altogether, these data confirm the major interest of the dechlorination of polychlorinated
- 391 compounds by ISCR for the limitation of CLD-induced endothelial dysfunction as angiogenesis
- and globally to prevent the CLD-induced human toxicity.
- 393

394 CONCLUSION

- 395 CLD dechlorinated derivatives CLD-3Cl and CLD-4Cl do not have pro-angiogenic effects *in*
- 396 *vitro* and *in vivo* and do not significantly increase prostatic tumor growth in a mouse xenograft
- 397 model. Consequently, the present study suggest, on one hand, the previous *in vitro* data
- 398 concerning the prevention of the pro-angiogenesis effects by dechlorination of CLD (Legeay et
- al. 2017) and, on the other hand, that *ISCR* should be considered as promising method for the
- 400 decontamination of soils polluted by CLD.

401

402 CONFLICTS OF INTEREST

403 The authors declare no competing financial interests.

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