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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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**KEYWORDS:** *In situ* chemical reduction, chlordecone, dechlorination, angiogenesis, prostate cancer

## ABSTRACT

*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 association between the development of prostate cancer and CLD exposure (Multigner et al. 2010). In a previous study, we demonstrated that the two main CLD derivatives obtained by the reduction method: CLD-1Cl and CLD-3Cl have lower cytotoxicity and proangiogenic properties than CLD itself (Legeay et al. 2017). By contrast, nothing is known on the 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.

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

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.

Evolution of prostate tumors growth was lower in the groups treated by the three metabolites compared to the one treated by CLD. The vascularization measured on the tumor sections was inversely proportional to the rate of dechlorination, the treatment with CLD-4Cl showing no difference with control animals treated with the vehicle oil used for all substances tested.

We can therefore conclude that pro-angiogenic effect of the CLD is significantly decreased following the ISCR-resulting dechlorination, and that the carcinogenic effect is reduced. Further investigations are now needed to elucidate the molecular mechanisms by which dechlorination of CLD reduces its carcinogenic and proangiogenic effects in prostate tumor.

# INTRODUCTION

Chlordecone (CLD, a.k.a. Kepone, decachlorooctahydro-1,3,4-metheno-2H-cyclobuta[c,d]pentalen-2-one, CAS No: 143-50-0) is a chlorinated hydrocarbon insecticide, 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 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 chronic food contamination and accumulation in liver, muscles, and fat of animals (Clostre et 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 bound to last for centuries if nothing is done to lower soil contamination (Cabidoche et al. 2009). Such persistence in the environment is a major health problem. Indeed, several studies have previously reported that chronic exposure to CLD leads to hepatotoxicity and increases the risk of cancer in rats (Reuber 1979; Mehendale 1981; Guzelian 1982; Dalu and Mehendale 1996; Murali et al. 2004). In Human, it significantly increases the risk of prostate and liver cancers, and pregnancy complications (Reuber 1979; Sirica et al. 1989; Faroon et al. 1995; Multigner et al. 2010; Dallaire et al. 2012; Kadhel et al. 2014; Multigner et al. 2016). Furthermore, previous study has reported that CLD is able to enhance *in vitro* angiogenesis through the activation of estrogen receptors, thus favoring both tumor growth and development (Clere et al. 2012).

*In situ* chemical reduction (ISCR) may be a solution for the remediation of soils contaminated 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 effective in remediation of soils contaminated by pesticides (Phillips et al. 2004, 2005, 2006; Kim et al. 2010). Among all the metabolites, only the major transformation products, *i.e.* a mono dechlorinated CLD: chlordecone - 1Cl (CLD-1Cl) and chlordecone - 3Cl (CLD-3Cl) were commercially available and tested. The CLD-1Cl and CLD-3Cl (the data available do not enable the position of dechlorination to be more precisely defined (Belghit et al. 2015)) represents 50 % and 13% respectively of the cumulated relative peak areas of the eleven transformation products identified after the 27-day ISCR treatment of an alluvial soil concerning the hypothetic malignant effects of these metabolites (Mouvet et al.). A third

derivative even more dechlorinated, quadri-hydro CLD (CLD-4Cl) is also observed, with a much lower relative area than CLD-3Cl.

Recently, we have demonstrated from *in vitro* data, that CLD-1Cl and CLD-3Cl have lower proangiogenic properties compared to CLD (Legeay et al. 2017). While the *in vivo* effects of CLD-1Cl and CLD-3Cl and their properties on tumor growth remain unknown, the aims of this study were to evaluate the influence of CLD and its metabolites in the control of neovascularization in a mice model of prostate cancer.

# MATERIALS AND METHODS

## *Reagents and chemicals*

CLD, purity 99.5%, was purchased from Dr. Ehrenstorfer (reference n12291). CLD-1Cl, CLD-3Cl and CLD-4Cl formed by ISCR were synthesized by Alpha-Chimica (Alpha-Chimica, Châtenay-Malabry, FRANCE) with a purity of 97.2%, 97.4% and 95.8%, respectively. GC-MS/MS shows the impurities to be other CLD dechlorinated derivatives. PC-3 cells (CRL-1435) were obtained from ATCC (Manassas, VA). RPMI 1640 medium, F-12 nutrient mix, penicillin-streptomycin, fetal bovine serum (FBS), were purchased from Invitrogen (Carlsbad, CA). Anti-CD31 antibody was purchased from (BD Biosciences). Biotinylated second antibody IgG was purchased from (Vector BA-9401). ECM gel® was purchased from (Sigma-Aldrich). CYQUANT® direct cell proliferation kit was purchased from (Invitrogen®).

## *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 heat-inactivated 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 L-glutamine, 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.

## *Chlordecone and metabolite dose calculations for in vivo experiments*

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

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

### *Xenograft model*

PC-3 cell suspension ($1.5.10^6$ cells in 150 µL PBS w/o FBS) was xenografted subcutaneously into the right flank of 5-weeks-old male *nude* mice (Envigo, Venray, N.L.). All animal procedures listed in this article were performed as per the protocol approved by the local ethic committees (Angers, France; protocol APAFIS #5872) and the number of animals was limited regarding animal ethics.

The 41 mice were then divided randomly into nine groups. Eight groups were exposed to CLD or its derivatives respectively at 33 µg/kg and at 1.7 µg/kg of body weight by daily gavage in 200 µL alimentary virgin olive oil for 8 weeks. The control group received daily gavage with 200 µL alimentary virgin olive oil for 8 weeks. The experimental plan followed is described in figure 1.

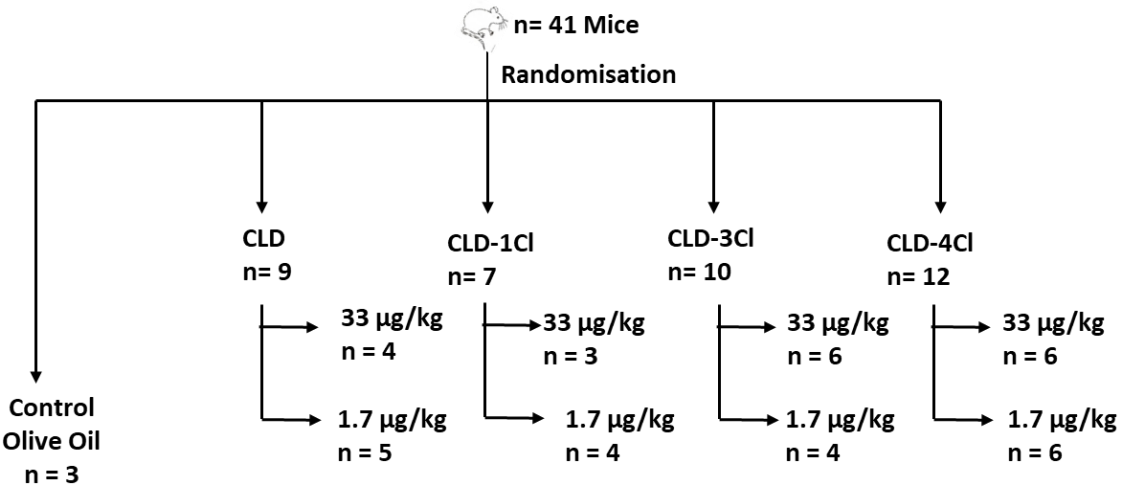



**Figure 1: Distribution of animals according to the conditions of exposure to different compounds.**

Tumor volume has been measured each 2 days according to the formula:

Tumor volume =[1⁄2(length x width²)] (Euhus et al. 1986).

Mice were sacrificed after 8 weeks and tumors were measured, dissected, weighed, and snap frozen in Tissue-Tek® O.C.T. compound and stored at -80°C for further analysis.

### *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 pg/µL) was added to 50 µL of mouse serum. Fat and proteins were then precipitated with 50µl formic acid by vortexing for 10 sec. Supernatants were loaded onto a C18 SPE cartridge, washed with hexane, eluted with acetonitrile and dried under a nitrogen stream. Dried extracts were reconstituted in 25 µL of a water/acetonitrile mix and injected into the LC-ESI-MS/MS instrument (Acquity-XevoTQS, Waters). Molecules were separated using an Accucor C30 column (Thermo) with a water/acetonitrile gradient. Targeted compounds were analyzed in SRM mode, except for chlordecol in SIM mode. The calibration range was from 0.5 to 50 pg/µL 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%.

### *Immunochemistry and vessel area quantification*

Tumor sections (7µm) 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 µg/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.

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

### *In vitro capillary network formation on ECM gel®*

HUVECs were seeded with a density of $10^4$ cells per well previously covered with ECM gel® (Sigma-Aldrich). Briefly, 10 µL 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 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 of angiogenesis.

### *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 PC-3 (ATCC®, CRL-1435) was analyzed using CYQUANT® assay kit (Invitrogen®). $5.10^3$ cells were seeded into a 96-well plate for 24 h and then were treated with $10^{-6}$M and $5.10^{-8}$M CLD, CLD-1Cl, CLD-3 and CLD-4Cl at concentrations (these reflect the target serum 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 suppressor 1) to 100µL of culture medium per well and the cells were incubated at 37°C for 60 min. Fluorescence levels were read by a fluorescent microplate reader (Clariostar) with a filter at 508 nm for excitation and 527 nm for emission.

### *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.

# RESULTS

1. 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 CLD or its derivatives.

In low dose groups (1.7µg/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)**. No significant difference of tumor growth was observed in mice treated with CLD-3Cl and CLD-4Cl compared to control mice.

In high dose groups (33 µg/kg), a significant increase of tumor growth has been observed in mice treated with CLD in comparison with control animals (p=0.01). No significant difference was observed between mice treated with CLD and mice treated with CLD-1Cl while tumor 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 difference was observed between CLD-3Cl-treated mice and CLD-4Cl-treated mice compared to control mice.

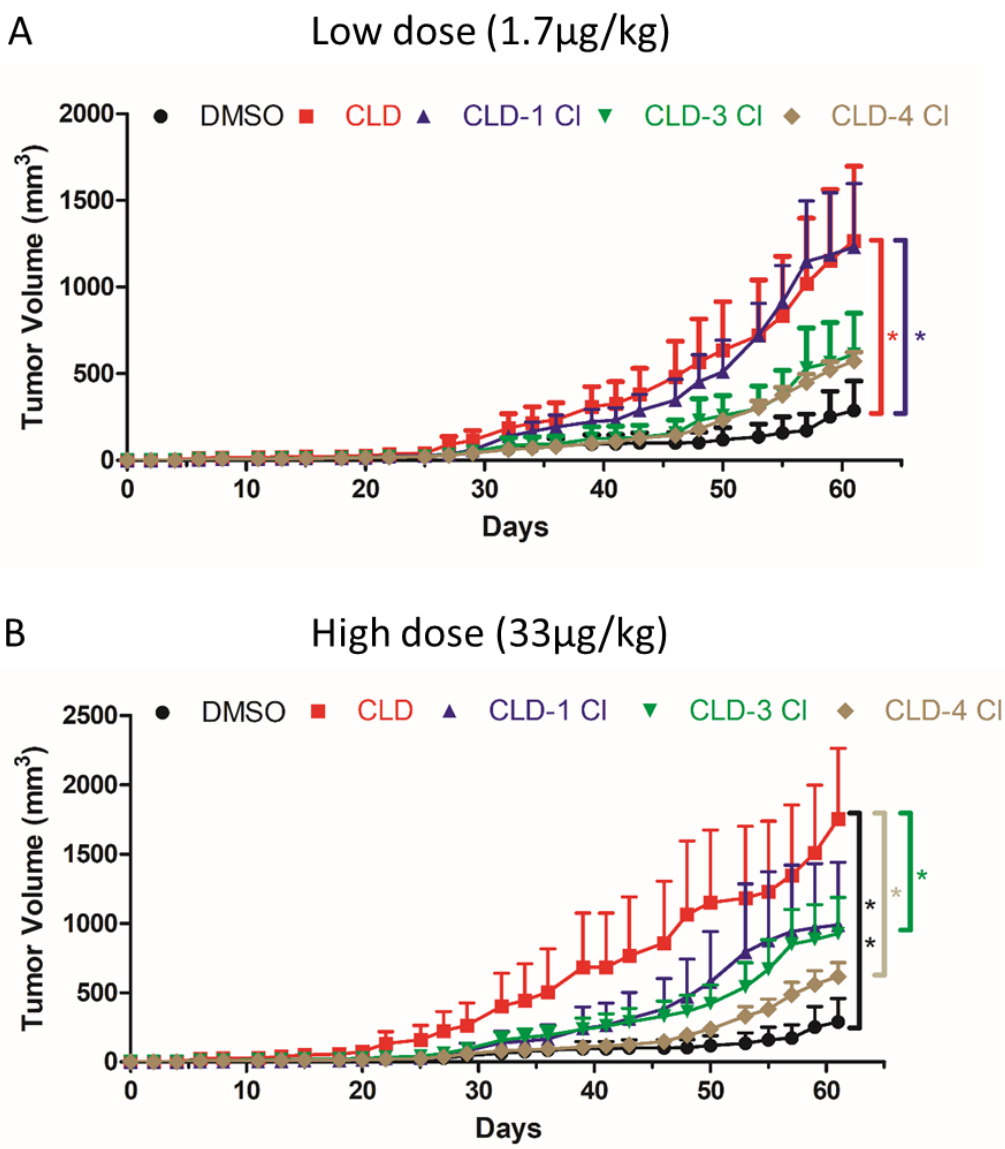



**Figure 2: Daily treatments for 8 weeks at low (1.7 µg/kg) and high (33 µg/kg) dose with CLD but not with CLD-3Cl and CLD-4Cl increase tumor growth formed by PC-3 cells in mice compared to control mice. *p<0.05; **p<0.01. n=3-12.**

2. Serum dosage of chlordecone and its dechlorinated derivatives

After the low dose exposure of CLD (1.7µg/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.

These serum dosages also reveal that for a same dose, chronicle ingestion of all dechlorinated derivatives leads to much less serum concentrations compared to CLD following the order of dechlorination: CLD > CLD-1Cl > CLD-3Cl > CLD-4Cl.

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, 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).

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

| | Concentration, mean ± SD (µg/L) | | | |
|---|---|---|---|---|
| | CLD | CLD-1Cl | CLD-3Cl | CLD-4Cl |
| CLD 1.7µg/kg | 26.3 ± 12.7 | | | |
| CLD 33µg/kg | 192.8 ± 73.6 | | | |
| CLD-1Cl 1.7µg/kg | | 11.9 ±3.2 | | |
| CLD-1Cl 33µg/kg | | 67.1 ± 21.2 | | |
| CLD-3Cl 1.7µg/kg | | | 4.0 ± 3.5 | |
| CLD-3Cl 33µg/kg | | | 6.1 ± 4.3 | |
| CLD-4Cl 1.7µg/kg | | | | < LOQ |
| CLD-4Cl 33µg/kg | | | | 2.2 ± 0.9 |

3. Effect of chlordecone and its dechlorinated derivatives on the proliferation of PC-3 tumor cells

No difference was found in PC-3 cell proliferation whatever the concentration and the type of dechlorinated CLD derivative (**Fig 3**).

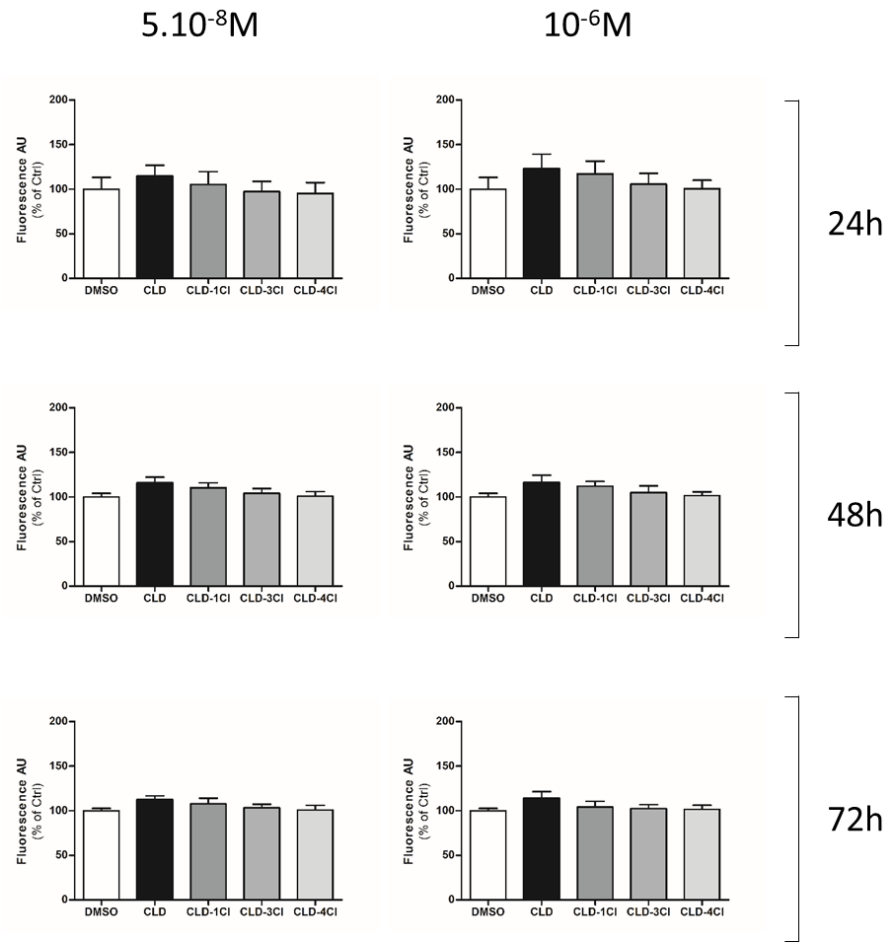
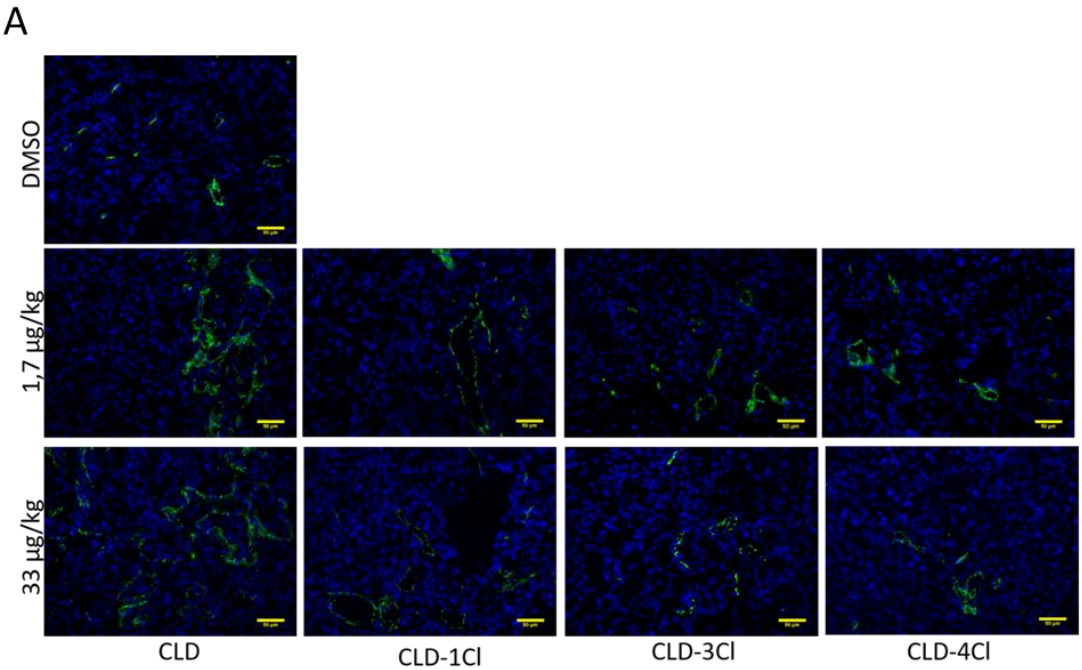
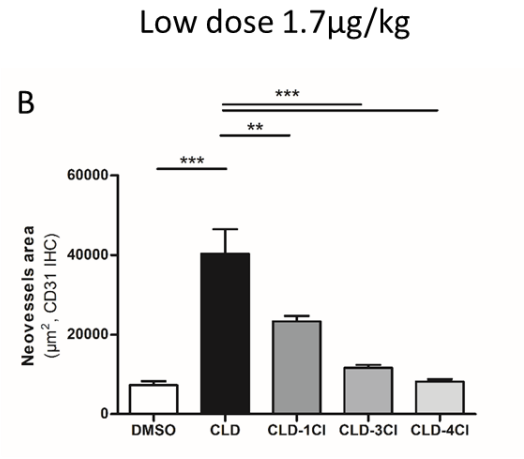



**Figure 3: Chlordecone or its derivatives even after 72h of exposure do not increase the proliferation of PC-3 tumor cells. n=6.**

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 µg/kg and p=0.0009 for 33 µg/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).



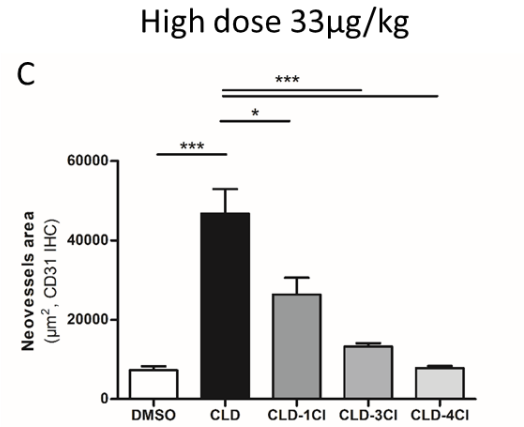




**Figure 4: Dechlorination of CLD decreases the tumor neovascularization. A) Immunolabling of tumor sections. In blue the labeling of cell nucleus with DAPI and in**

**green the labeling the endothelial marker CD31. (Scale 50 µm). B and C) Quantification of the neovascularization. *p<0.05, **p<0.01, ***p<0.001. n=6**

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

*In vitro* assays performed in a previous study suggested that CLD-1Cl and CLD-3Cl have lower proangiogenic properties than the CLD parent molecule on human primary endothelial cells (Legeay et al. 2017). Because the present study has an additional derivative, CLD-4Cl, 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 concentrations $5.10^{-8}$ M (concentration that has been found in plasma of patients exposed), $10^{-11}$ M (concentration that has been found in drinking water) and $10^{-9}$ M (intermediate concentration) for 24h. It has been found that CLD-4Cl does not significantly increase capillary formation *versus* control cells for the three concentrations tested ($5.10^{-8}$ M, $p = 0.064$; $10^{-9}$ M, $p = 0.063$; $10^{-11}$ M, $p = 0.1$). Additionally, it has been observed that CLD-4Cl possesses a significantly lower proangiogenic effect than CLD ($5.10^{-8}$ M, $p = 0.004$; $10^{-9}$ M, $p = 0.003$; $10^{-11}$ M, $p = 0.009$) (**Fig 5 and Table 2**).

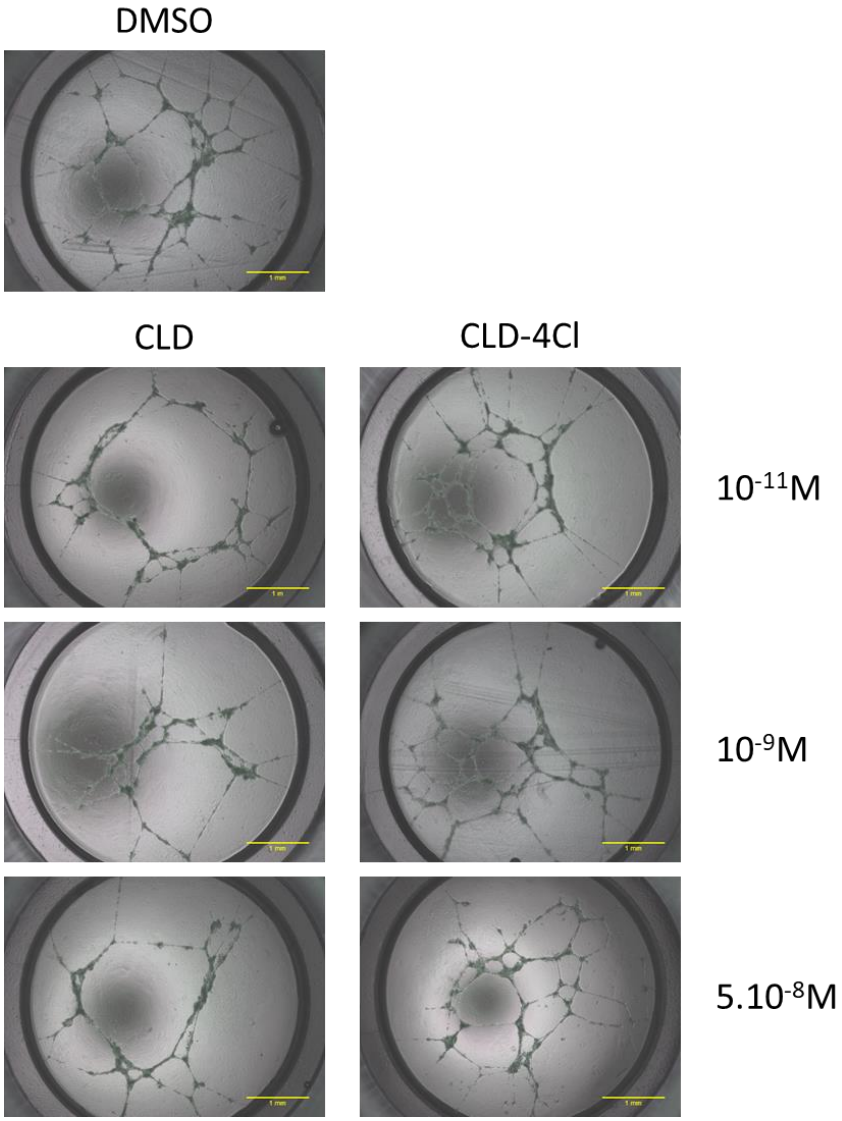



**Figure 5: CLD but not CLD-4Cl significantly increases capillary formation of HUVECs in culture on ECMgel® after 24h of treatment. Scale=1mm.**

**Table 2: Quantification of the capillary area formed by HUVECs in percentage of control**

| | CLD | CLD-4Cl |
|---|---|---|
| $10^{-11}$ M | 196.00 ± 20.85[a] | 123.00 ± 10.39** |
| $10^{-9}$ M | 196.00 ± 10.53[a] | 122.17 ± 7.53** |
| $5.10^{-8}$ M | 198.67 ± 13.77[a] | 122.83 ± 8.08** |

[a]$p<0.001$ vs ctrl

**p<0.01 vs CLD
n=6

# DISCUSSION

In order to confirm our previous *in vitro* data (Legeay et al. 2017), this study aimed to evaluate the proangiogenic properties of dechlorinated derivatives of CLD compared to CLD in a mouse model of tumor-xenograft and *in vitro*. In line with the literature, CLD potentiates prostatic tumor growth *in vivo* (Multigner et al. 2010) and this effect was associated with an increased neovascularization (Clere et al. 2012; Legeay et al. 2017; Alabed Alibrahim et al. 2018), confirming the proangiogenic effects of CLD. Prostate cancer is one of the most common cancer diagnosed in men and higher incidence rates have been observed in regions highly 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. 2016a, b; Deloumeaux et al. 2017) and any possible way of lowering permanently the CLD soil 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).

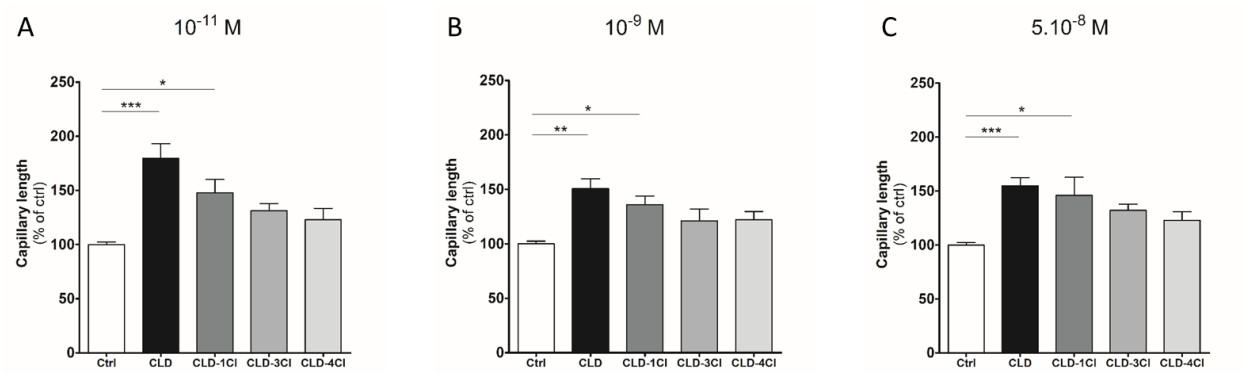



Figure 6: The levels of dechlorination leads to less or absence of pro-angiogenic effects compared to CLD. Combined data from the present study and Legeay S. *et al* (2017). *p<0.05; **p<0.01; ***p>0.001. n=6.

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 µg/kg and 33 µg/kg in order to reach 25 µg/L ($5.10^{-8}$ M), the serum concentration in industrial workers, and 500 µg/L ($10^{-6}$ M), the serum concentration after high human exposure respectively described with CLD (Adir et al. 1978; Cohn et al. 1978). The serum concentrations of CLD measured after 8 weeks was 26.7±12.7 µg/L which is very close to the targeted serum concentration and confirms our extrapolations for the pharmacokinetics of CLD in mice. However, for the highest dose 33 µg/kg, the serum concentration of CLD was 192.8±7.6 µg/L while the targeted serum concentration was 500 µg/L. This decrease of the observed serum concentration would be partly explained by a possible bioaccumulation in organs such as liver, fat or kidney, leading to a lower CLD concentration in the blood compartment as previously described after oral exposure to CLD (Cohn et al. 1978; Rochelle and Curtis 1994; Faroon et al. 1995). However, the same dose of dechlorinated CLD derivatives leads 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. These data suggest a decrease of the oral bioavailability or an increase of the metabolism or both of the CLD derivatives compared to CLD. This hypothesis should be verified by deeper pharmacokinetic studies. The presence of CLD and some of its derivatives at very low concentration in serum of treated mice might be due to the presence of impurities in the original product and/or be explained by cross-contaminations. However, because their concentrations were 5 to 175 times lower, they can be considered as negligible.

According to the two targeted serum concentrations 25 µg/L ($5.10^{-8}$ M) and 500 µg/L ($10^{-6}$ M), PC-3 cell proliferation has been evaluated after exposure to CLD and its derivatives with these both concentrations for 24h to 72h. In addition, the evaluation of the angiogenic process has 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 concentration described in exposed human and the CLD concentration found in drinking water (Adir et al. 1978; Landrigan et al. 1980; Taylor 1982; Badach et al. 2000) according to our previous study (Legeay et al. 2017). Altogether, these *in vitro* data confirm that even at very low concentration, CLD and CLD-1Cl but not CLD-3Cl nether CLD-4Cl have proangiogenic effects while no proliferation of PC-3 cell are observed, certifying that the *in vivo* tumor growth after chronicle exposure is at least due to a stimulation of angiogenesis process.

It is interesting to note that the lower proangiogenic and tumorigenic properties of dechlorinated derivatives compared to CLD are correlated with the level of chlorination. From *in vivo* data, 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*.

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

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

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

## CONCLUSION

CLD dechlorinated derivatives CLD-3Cl and CLD-4Cl do not have pro-angiogenic effects *in vitro* and *in vivo* and do not significantly increase prostatic tumor growth in a mouse xenograft model. Consequently, the present study suggest, on one hand, the previous *in vitro* data 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 decontamination of soils polluted by CLD.

## CONFLICTS OF INTEREST

The authors declare no competing financial interests.

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