Localization and *in situ* absolute quantification of Chlordecone in the mouse liver by MALDI imaging

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Supporting Information.

- Method used for matrix deposition with the spraying device
- Method used for the quantification of chlordecone in the mouse liver by gas chromatography
- Figure S1: Histological images of liver sections from control mice and mice exposed to chlordecone, CCl_4 and chlordecone + CCl_4.
- Figure S2: Measurements of Alanine Transaminases reflecting the level of liver necrosis in the different mice.

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Method used for matrix deposition with the spraying device

Different matrices were tested to optimize the detection sensitivity of chlordecone and the best results were obtained with DCTB. However, this matrix is known to sublimate rapidly under the vacuum of the MALDI ionization source. Therefore, the solvent composition of the matrix solution was optimized to increase the stability time of the matrix layer. With a solution of DCTB at 7 mg/mL prepared in acetone/ACN/H_2O (60/30/10, v/v/v) we achieved matrix stabilization for 3 hours. The important part of organic solvent in the matrix solution was not compatible with the standard protocols of matrix deposition available in the ImagePrep device. Thus, the method for matrix deposition had to be specifically adapted: after an initialization step consisting in 15 cycles with a spray power at 15%, an incubation time of 5 sec and a drying time of 35 sec, 13 cycles were performed under sensor control with a final voltage difference at 0.20 V, a spray power at 20%, an incubation time of 30 sec and a drying time under sensor control at 20% and a safe dry of 50 sec.

Method used for the quantification of chlordecone in the mouse liver by Gas Chromatography

All mouse liver samples were freeze-dried with a Benchtop 3L Sentry Lyophilisator (VirTis, New-York, USA). Extraction of chlordecone was performed with a mixture of n-hexane/dichloromethane (90/10, v/v) using an Accelerated Solvent Extractor ASE (Dionex 200, Sunnyvale, USA). Before the extraction, surrogate PCB congener 112 (Dr. Ehrenstorfer®, Augsburg, Germany) was added at a final concentration of 50 pg/µL to the samples in order to quantify possible loss of chlordecone during the extraction and purification procedure. Solvent was evaporated using a Turbovap LV (Zymarck, Hopkinton, Mass., USA) until a constant weight was obtained. Samples were then diluted in 3 mL *n*-hexane. The extracts were subjected to clean-up with 2 mL of sulphuric acid (98-100%)

(Merck, Darmstadt, Germany) in order to remove organic matter (lipids, lipoproteins, glucides). The addition of sulfuric acid is also known to convert chlordecone hydrate into chlordecone.[1] Thus, in our conditions, GC quantifies the global quantity of chlordecone (i.e., chlordecone+chlordecone hydrate). The samples were then homogenized by vortexing with a Vibramax 110 (Heidolph, Germany) before being centrifuged for 3 min at 2160G at 10°C with a JOUAN BR4i centrifuge (Jouan, St-Nazaire, France). The organic phase was transferred to another tube and the acidic phase was extracted with 3 mL of n-hexane, vortexed and centrifuged for 3 min. The organic layers of subsequent centrifugations were pooled.

The elution was evaporated under a gentle stream of nitrogen just to dryness using a Visidry evaporator (Supelco, Sigma-Aldrich, St-Louis, USA). 50 µL of n-hexane and 50 µL of PCB 209 (100 pg/µL diluted in n-hexane (Dr. Ehrenstorfer®, Augsburg, Germany)) were added to each sample prior to the analysis by HRGC ^{63}Ni ECD. The samples were analysed for chlordecone by high-resolution gas chromatography (HRGC) using a Thermo Quest Trace 2000 gas chromatograph equipped with a Ni^{63} ECD detector (Thermo Quest, Milan, Italy) and an autosampler for liquids Thermo Quest AS 2000 (Thermo Quest, Milan, Italy). The analytical parameters were described elsewhere[2]. The average extraction yield of chlordecone from mouse livers was of 78%.

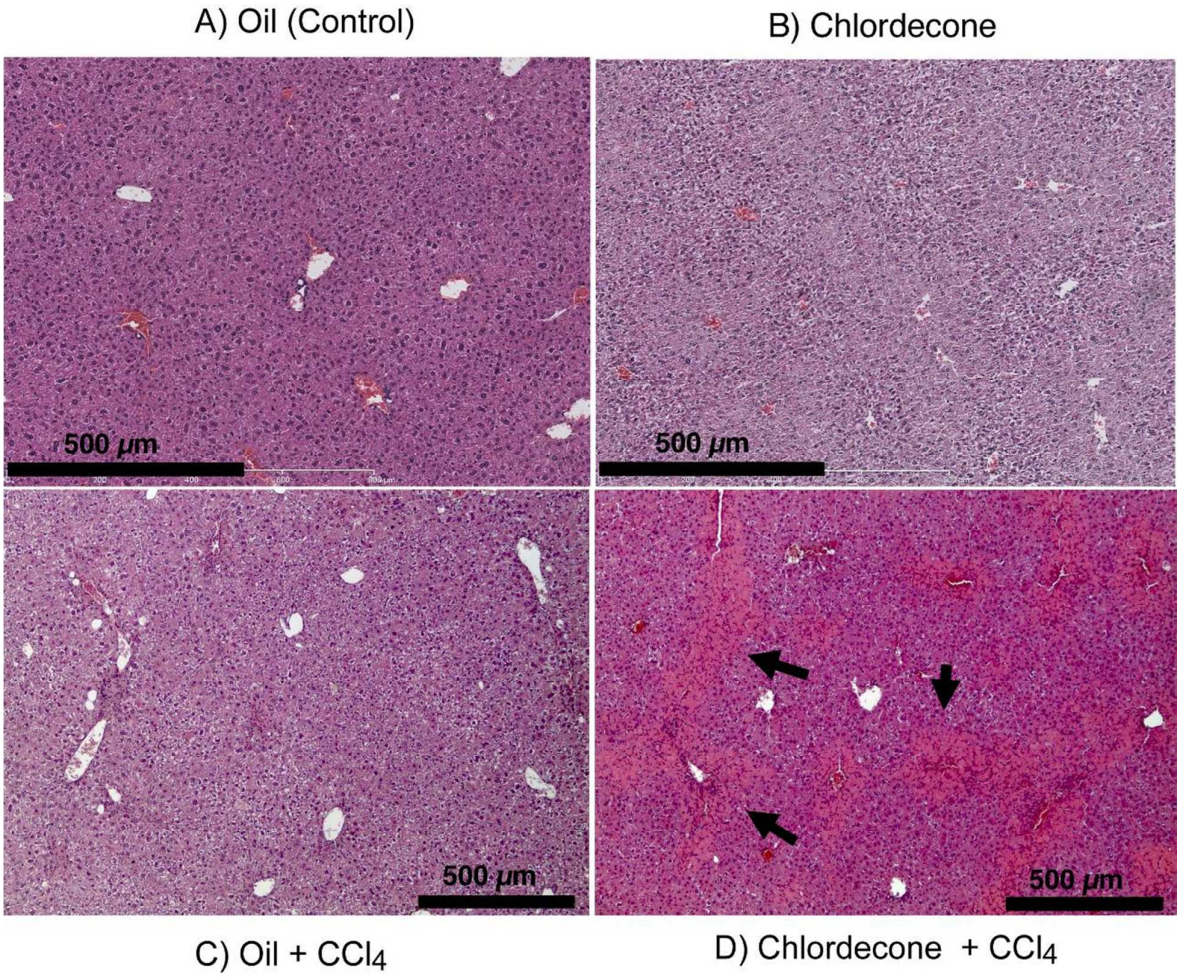

Figure S-1: Section of livers (paraffin embedded) from mice treated by gavage with **A)** oil (control), **B)** chlordecone alone, **C)** CCl_4 alone in oil or **D)** chlordecone + CCl_4 were stained with H&E for histopathology. Zone of liver injury are marked by arrows. Scale bar was 500 µm. The histopathological analysis of livers was performed as recently reported[3].

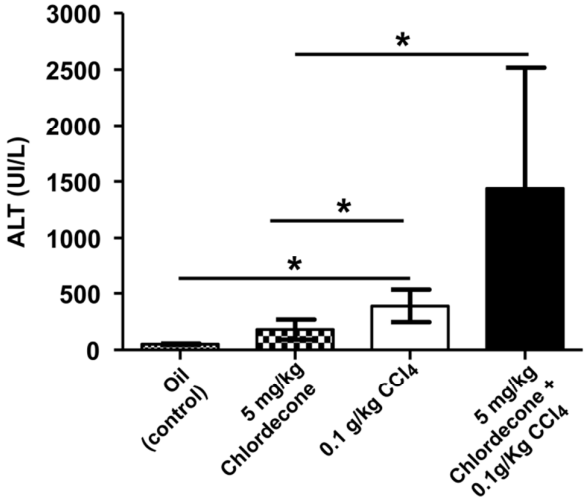

Figure S-2: Serum alanine transaminase (ALT) from mice were assayed according to the IFCC primary reference procedures and using the Olympus AU2700 Autoanalyser® (Olympus Optical). The results in each model are represented as means ± standard error of the mean (SEM) of each group. Mann-Whitney *U* test was used for comparison of control group parameters with treatment group and multiple group analysis was carried out by one-way ANOVA with post Mann-Whitney *U* test as reported earlier[3]. The correlation between continuous variables was analysed by using GraphPad Prism5 software. For all statistical analyses, * $p<0.05$, ** $p<0.01$ and *** $p<0.001$. The serum biochemical analysis of livers was performed as recently reported[3].

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