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Computational Approaches for Localization and Quantitation of Compounds, Toxicants and their Metabolites

Study setup

Model A
Chlordecone-treated liver

Time of exposure to chlordecone (days)

Model B
Chlordecone+CCl4-treated pathological liver (liver necrosis)

- Chlordecone 8 mg/kg bw
- CCl4 0.1 mg/kg bw
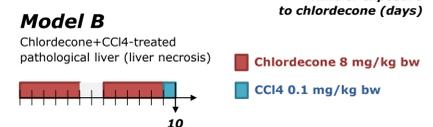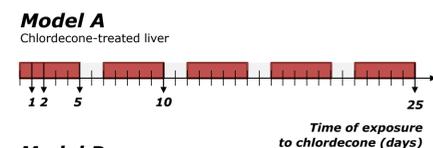
**Absolute quantitation in MALDI mass spectrometry imaging:**
- **Model A**: Healthy mice exposed to chlordecone, 5 mg/kg, for 1 to 25 days
- **Model B**: Mice presenting liver necrosis were exposed to chlordecone, 8 mg/kg, for 10 days then CCl4 at 0.1 mg/kg
- Control livers were from mice subjected to the same delivery, daily gavage with olive oil, but without chlordecone.

Chlordecone hydrate mass spec:
- Chlordecone is readily converted in a chlordecone hydrate form that can be detected by MALDI mass spectrometry in the negative ionization mode at m/z 506.68

Chlordecone hydrate

Chlordecone $C_{10}Cl_{10}O$ — Chlordecone hydrate $C_{10}Cl_{10}O_2H_2$ — Chlordecone hydrate $[C_{10}Cl_{10}O_2H_2]^- = [M+OH]^-$ m/z 506.68
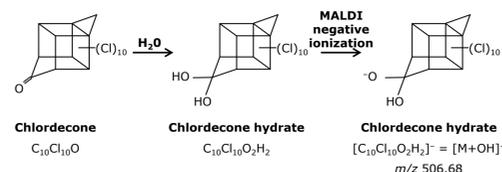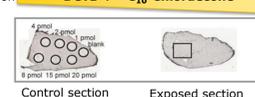
MALDI measurement:
- DCTB matrix including $^{13}C_{10}$-Chlordecone (8 pmol/µl)
- UltrafleXtreme (Bruker) negative reflector mode
- Spatial resolution 100 µm
- m/z range 400 – 1000
- Data analysis in SCiLS Lab 2019b

Matrix deposition — DCTB + $^{13}C_{10}$-Chlordecone

Control section — Exposed section
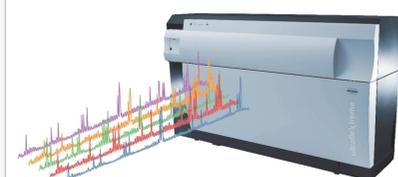
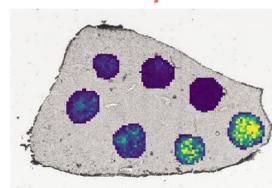**Quantitation of chlordecone:**
- Different amounts of chlordecone (from 1 to 20 pmol/spot) were manually spotted on a control section mounted next to an exposed section.
- Labelled normalization consisting in the normalization of the intensity of chlordecone hydrate detected at m/z 506.68 to the intensity of $^{13}C_{10}$-chlordecone hydrate detected at m/z 516.71 was then performed.
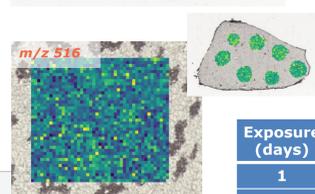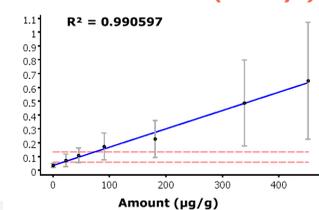
Absolute quantitation

Calibration of quantitation:
- The normalized intensity of the m/z 506.68 ion detected on each calibration spot was reported as a function of the amount of chlordecone deposited on the control section expressed in µg/g in order to calculate a calibration curve.
- Using the equation of the calibration curve, the normalized intensity of the m/z 506.68 ion detected on the exposed section was converted in a quantity of chlordecone expressed in µg/g.
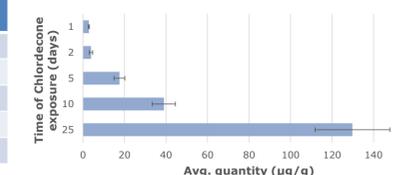
Internal standard:
- The uniform distribution of the internal standard (m/z 516.71) on the tissue section confirms that the differential distribution of chlordecone is not due to ion suppression effects.
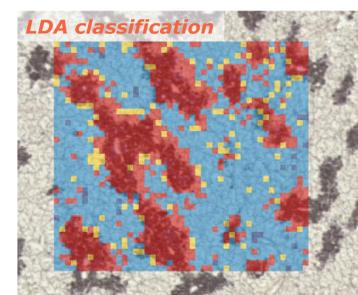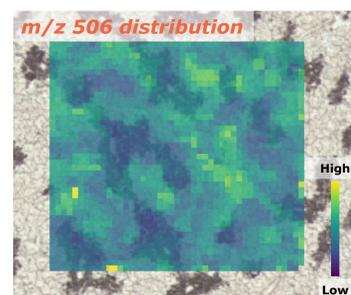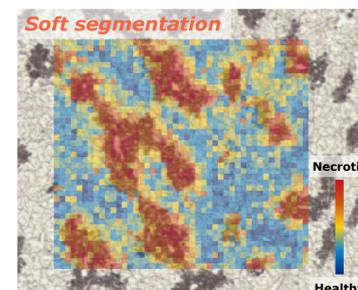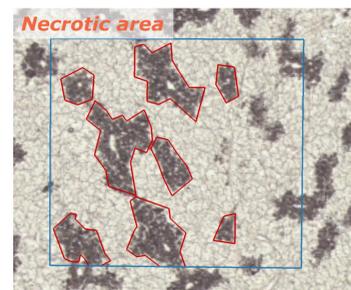
Calibration spots

m/z 516

Calibration curve (10 days)

$R^2 = 0.990597$

Amount (µg/g)

Quantity of chlordecone for different time points:
- Accumulation profiles of chlordecone (5 mg/kg bw) in the mouse liver depending on days.
- Low RSD of quantitation results reflect good reproducibility.
- Limit of detection (LOD) and quantitation (LOQ) estimated at 1.2 µg/g and 3.9 µg/g.

Exposure (days)	Avg. quantity (µg/g)	Std. Dev.	RSD%
1	2.9	0.3	10.3
2	3.9	0.8	20.5
5	17.6	2.6	14.8
10	39.0	5.6	14.4
25	129.8	18.0	13.8

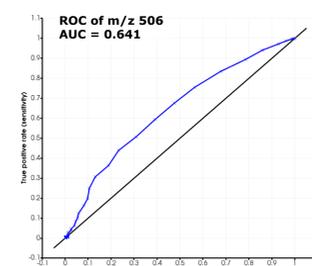Time of Chlordecone exposure (days) / Avg. quantity (µg/g)

Toxicant reaction

Absolute quantitation in pathological liver:
- Liver of a chlordecone and CCl4 treated mouse.
- Mouse exposed to chlordecone at 7 mg/kg bw for 10 days then to CCl4 at 0.1 mg/kg bw.

Average quantities:
Average in area: 83.5 µg/g
Average in healthy tissue: 91.2 µg/g
Average in necrotic tissue: 69.2 µg/g

ROC of m/z 506
AUC = 0.641

True positive rate (sensitivity) / False positive rate (1-specificity)

Necrotic area

Soft segmentation

Necrotic / Healthy

m/z 506 distribution

High / Low

LDA classification

Soft segmentation of MALDI imaging:
- Using annotated regions of necrotic area, a *linear discriminant analysis* (LDA) model was built with SCiLS Lab 2019b.
- Soft segmentation from LDA scores evaluate the probability for assignment to necrotic and healthy tissue.
- Quantitative evaluation is achieved by constructing histograms with kernel density estimate functions and *receiver operating characteristic* (ROC) curves.

- Necrotic tissue estimate
- Healthy tissue estimate

Log_{10} intensity m/z 506
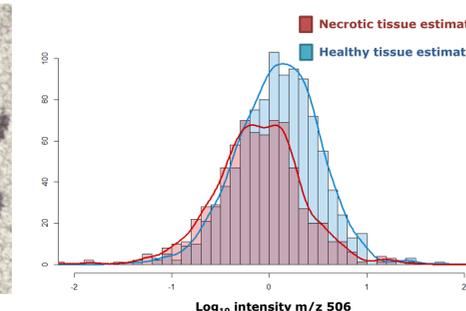
Background

- Chlordecone is an organochlorine pesticide that was extensively used in the French West Indies to fight weevils in banana plantations from 1973 to 1993.
- This has led to a persistent pollution of the environment and to the contamination of the local population for several decades with effects demonstrated on human health.
- Chlordecone accumulates mainly in the liver where it is known to potentiate the action of hepatotoxic agents.

Summary

- Mass spectrometry imaging (MSI) is widely used in drug development and toxicology studies to gain information about absorption and distribution of parent compound and its metabolites as well as how the presence of these compounds impacts distribution of endogenous molecules.
- Previous work by Lagarrigue et al. (Anal. Chem. 2014:86) presented an approach for quantitative mining MSI data of chlordecone toxicology study.
- The approach shown here includes methods for statistically expressing penetration in both qualitative and quantitative terms by soft segmentation, kernel density estimation, discriminant analysis, and quantitation of target molecules.

Highlights

- **Quantitation of small molecules by MALDI imaging** based on labelled normalization
- MALDI imaging reveals **in-situ localization of toxicant** in pathological liver preserving information of localization

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