Comparative study of the cytotoxic effects of microcystin-LR in mammalian cell lines: HepG2, Vero-E6, MDCK and Caco2.

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Environmental Health Department

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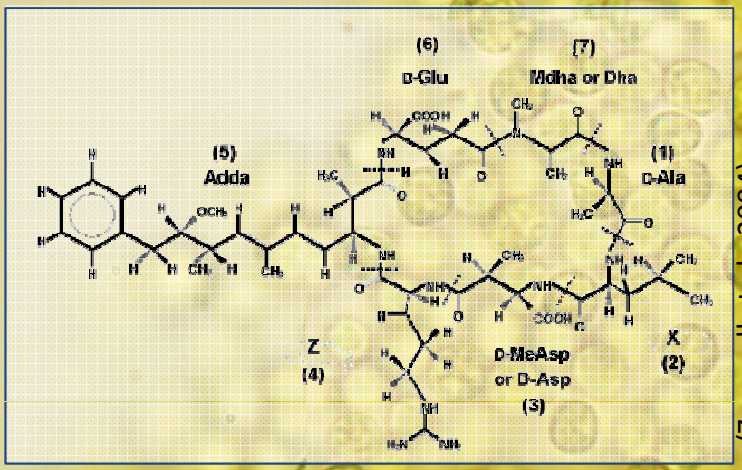
Microcystin-LR and *in vitro* cell lines



(Zurawell et al., 2004)

Hepatotoxin
PP1 and PP2A inhibition

Previous findings in Vero cells:
Low concentrations MCLR → autophagy, ER and lysosomes
(Alverca et al., 2009 and Dias et al., 2009)

1. Establish cytotoxicity tresholds and a dose-response relation for each cell line
2. Detect the intracelular targets of MCLR
3. Compare the effects of MCLR in the different cell lines

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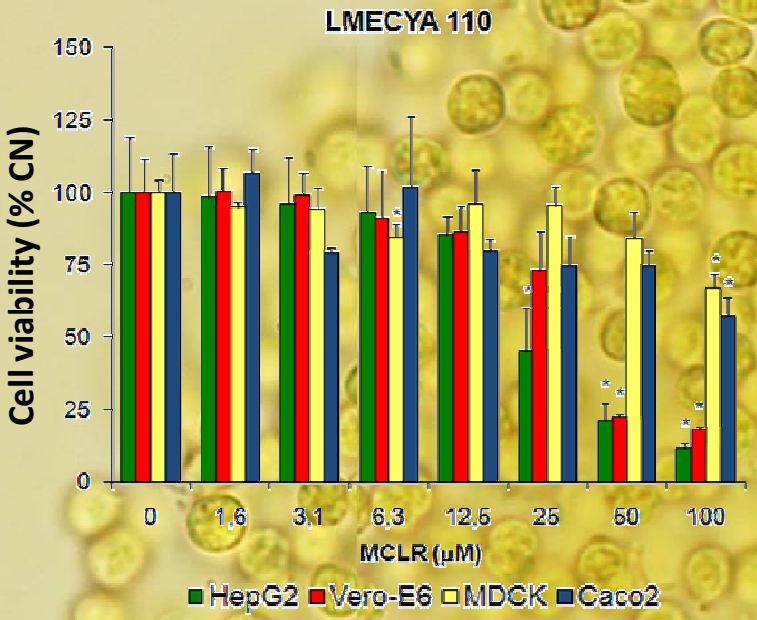
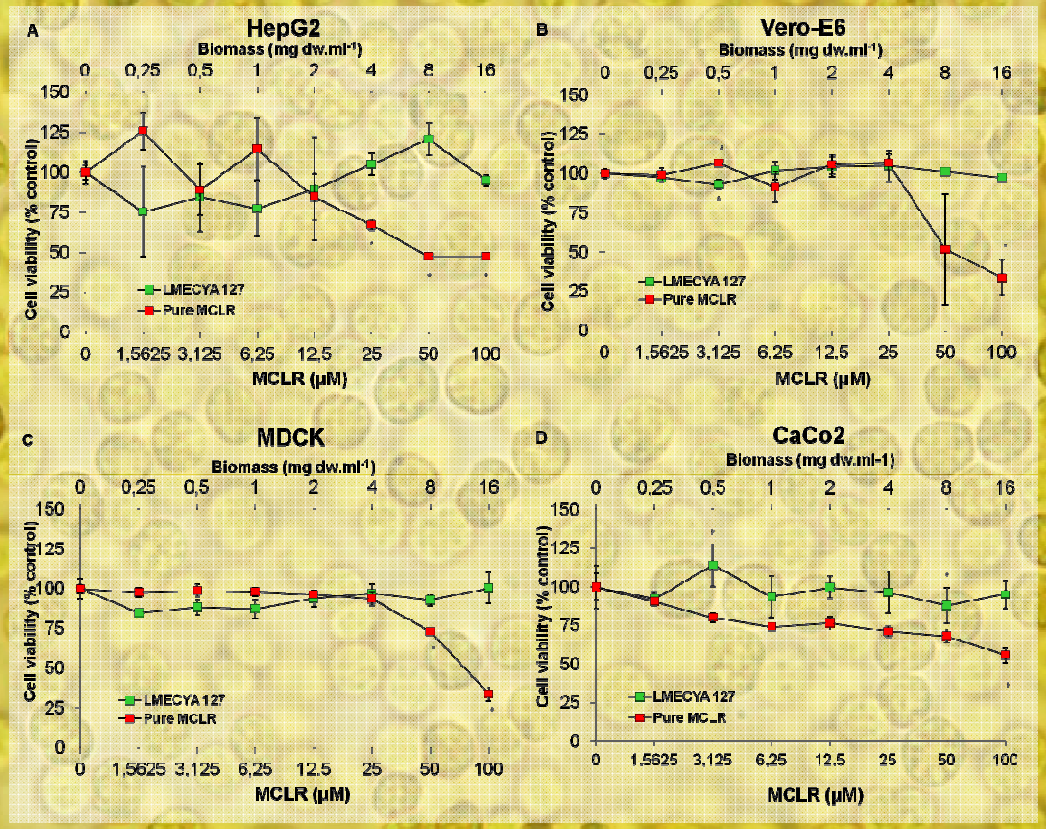
MCLR cytotoxicity

Neutral Red Uptake Test





HepG2 and Vero cells are more sensitive to MCLR toxicity

The effect of extract MCLR (LMECYA110) is similar to pure commercial MCLR

There is no effect of the matrix of the extract

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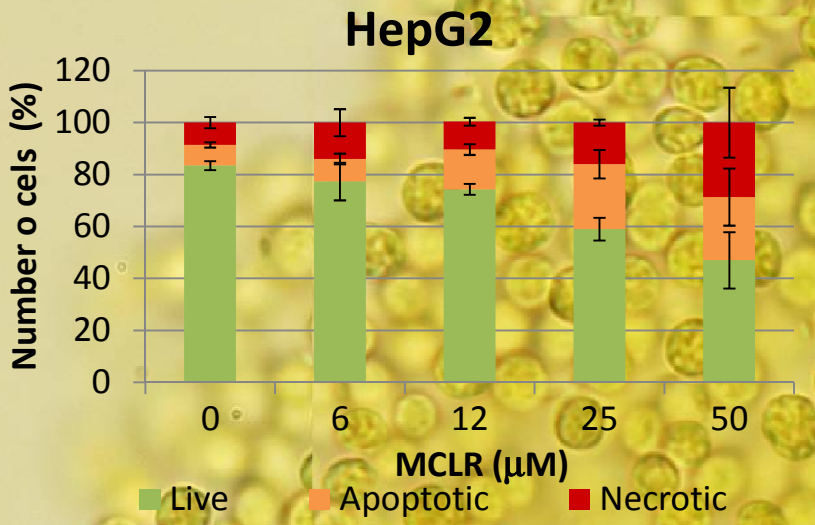
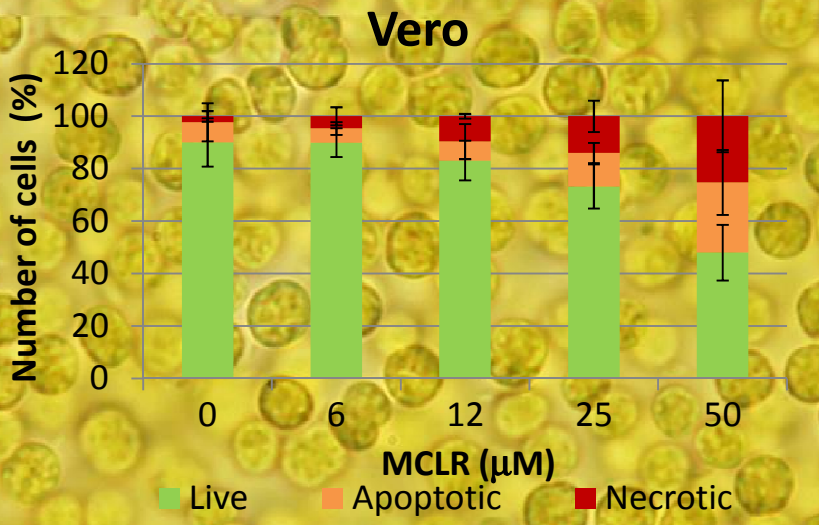
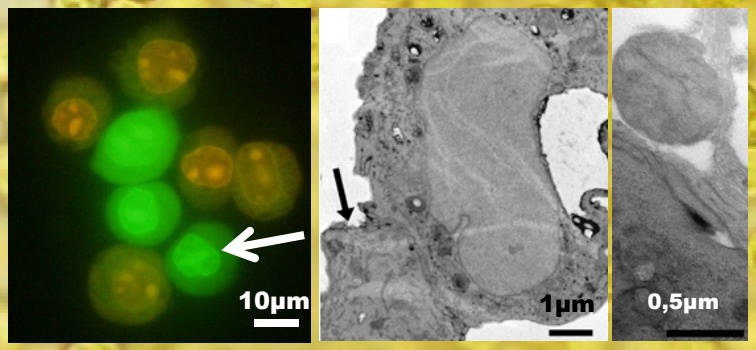
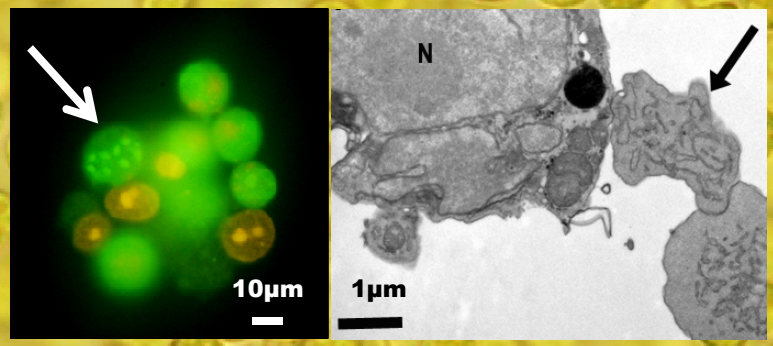
Cytotoxicity and apoptosis

Ethidium Bromide/Acridine Orange stainning









The decrease in cell viability is associated to an increase in the proportion of apoptotic and necrotic cells

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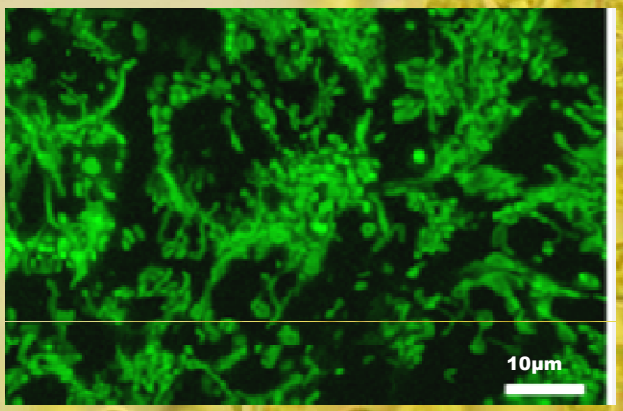
Intracellular targets of MCLR

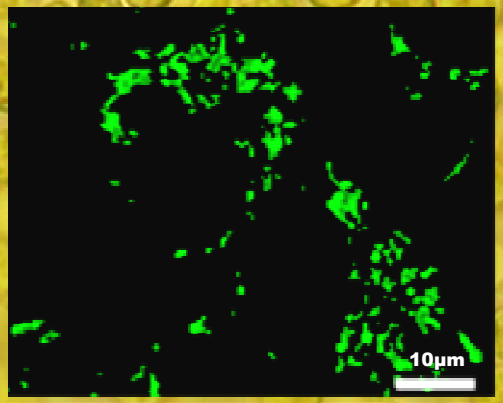
Mitochondria

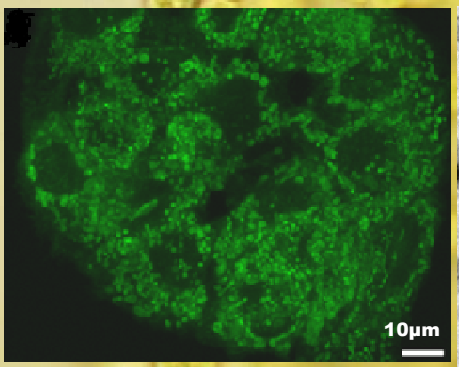
HepG2

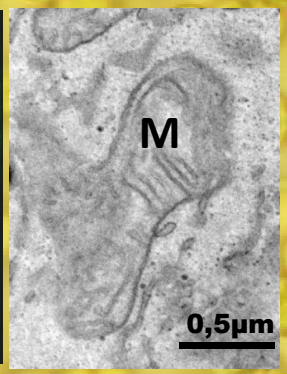
Vero



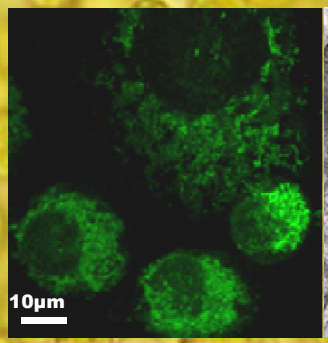
Control
Normal
morphology

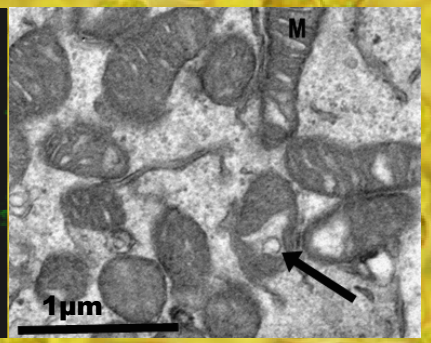






50 µM MCLR
Mitochondrial
disrupture





Mitochondria are affected at high MCLR concentrations

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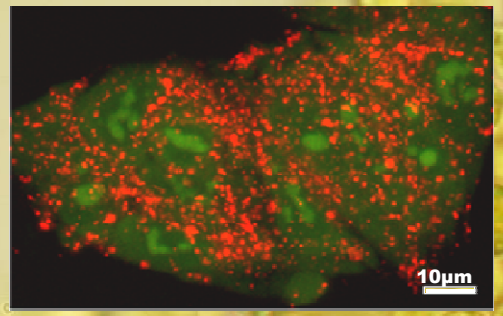
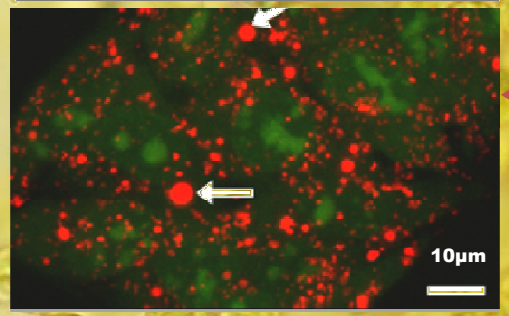
Intracellular targets of MCLR

HepG2 **Lysosomes** **Vero**



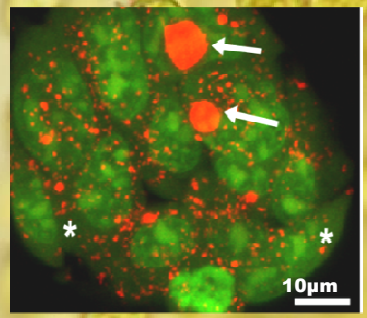
Control
Numerous lysosomes

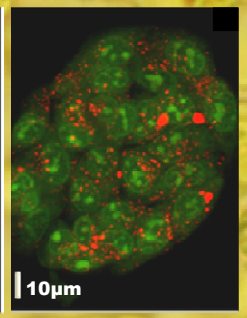


6 µM MCLR

Eniargement of some lysosomes

12 µM MCLR

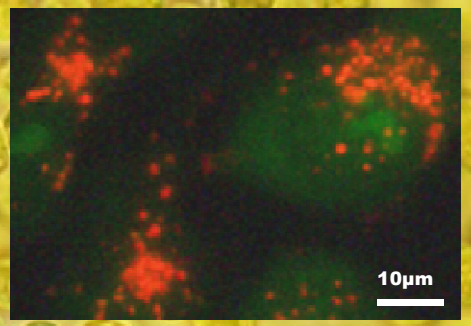


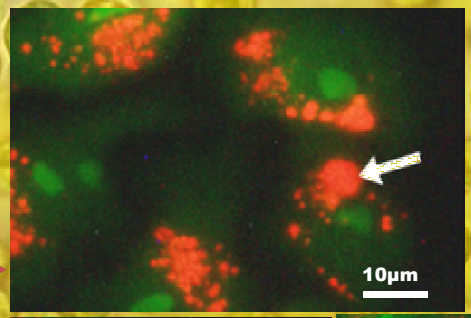


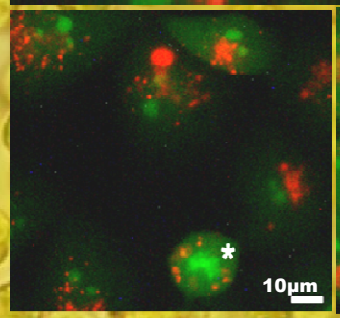
25-50 µM MCLR

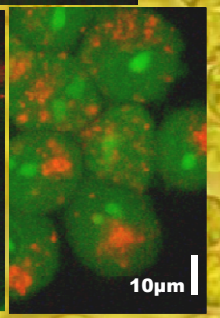
Rupture

Green fluorescence enhancement









Structural alterations in lysosomes begin at low MCLR concentrations

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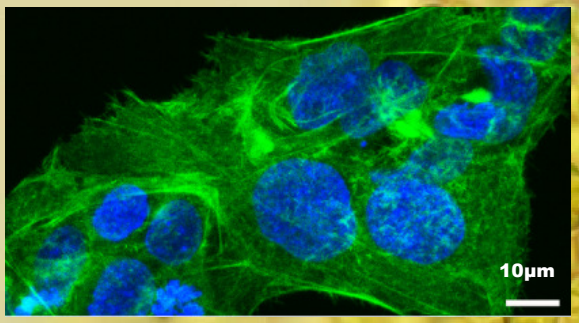
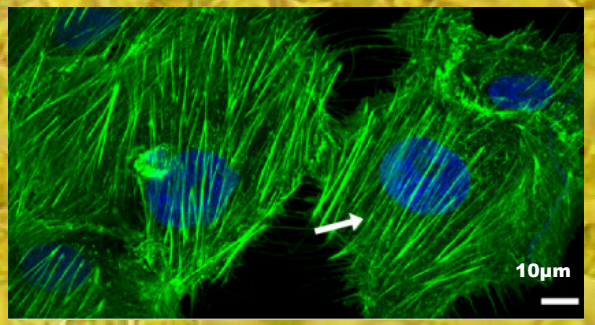
Intracellular targets of MCLR

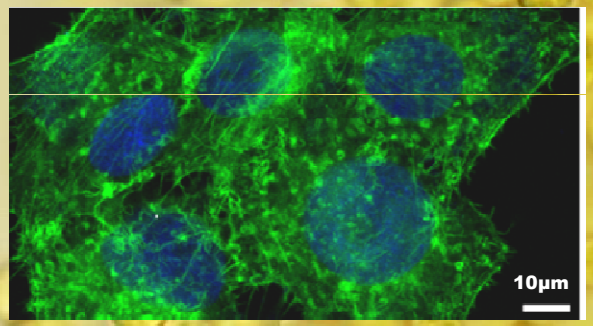
Actin cytoskeleton

HepG2 | Vero

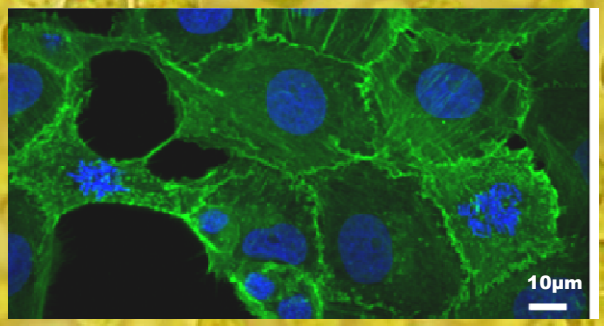


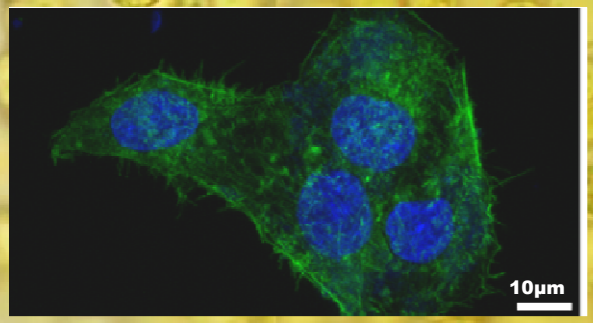
Control
Normal
distribution



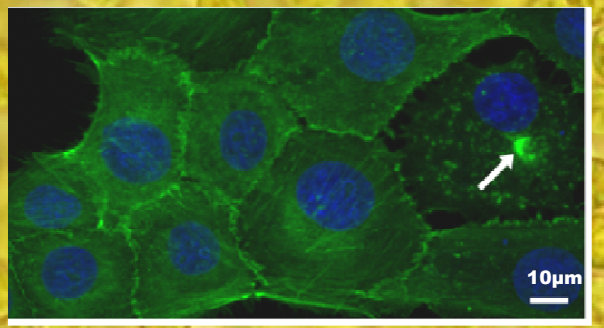


25 µM MCLR
Depolimerization





50 µM MCLR
Stress fibers
reduction



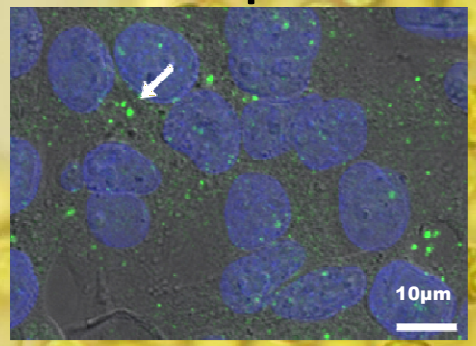
MCLR concentration dependent destabilization of actin fibers

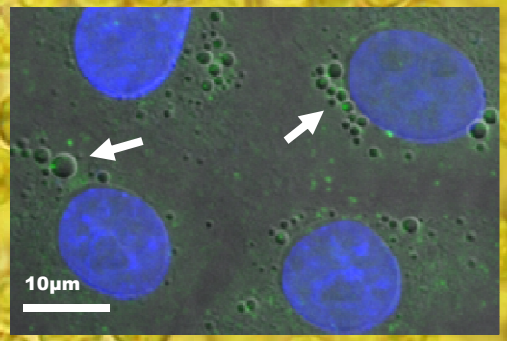
Intracellular targets of MCLR

Autophagosomes - LC3B protein

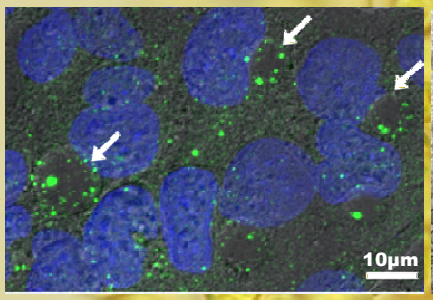
HepG2 | Vero

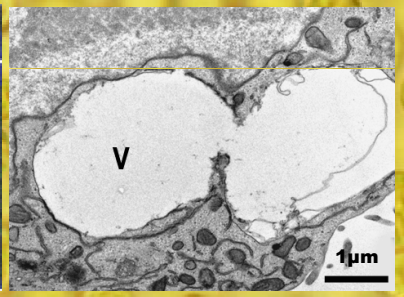
Control

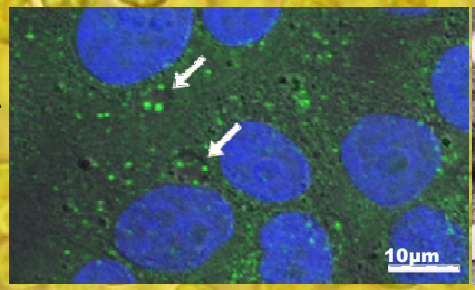


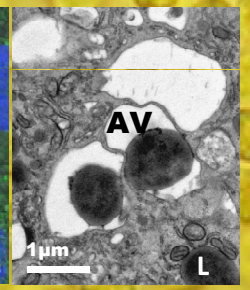


6-12 µM MCLR
Increase in autophagy

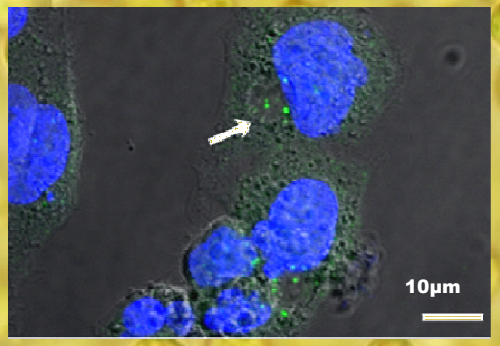


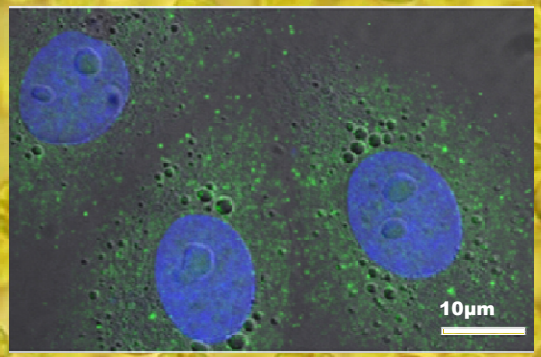






25 µM MCLR
Basal autophagy





Stimulation of autophagy at sub-cytotoxic MCLR concentrations

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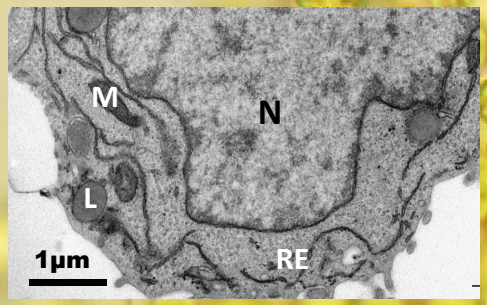
Intracellular targets of MCLR

Endoplasmic Reticulum ultrastructure

HepG2 **Vero**



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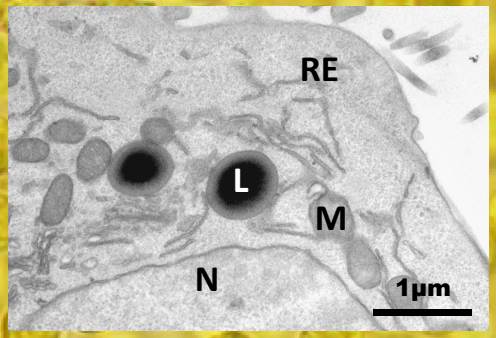
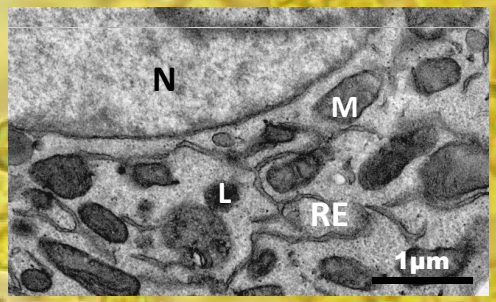
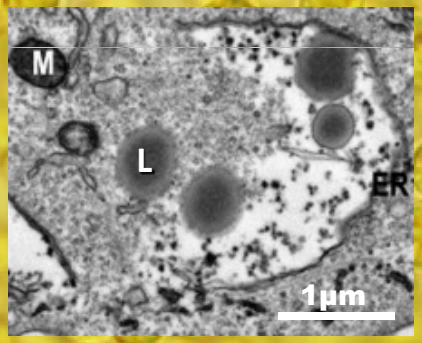
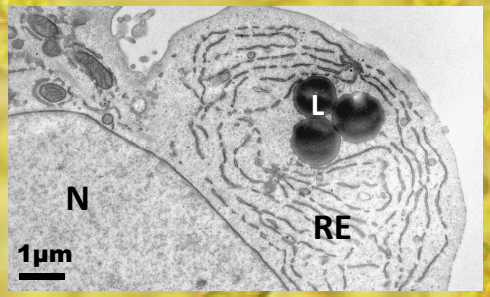


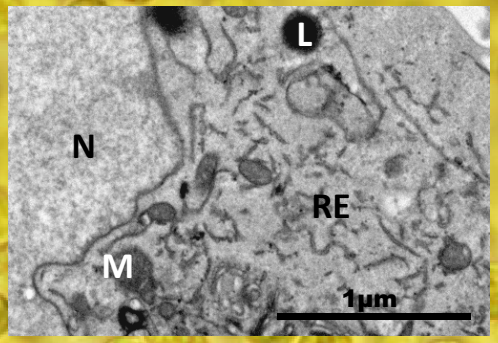


6 µM
ER vacuolization





50 µM
ER fragmentation



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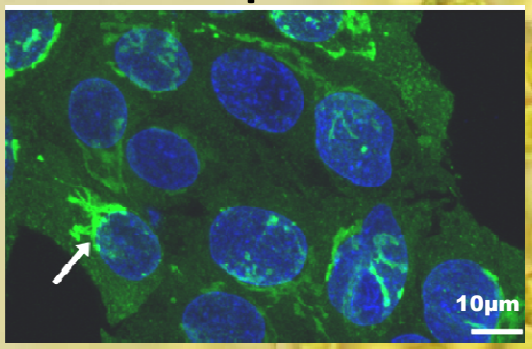
Intracellular targets of MCLR

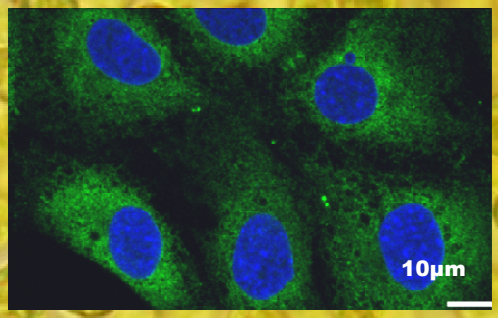
GRP94 ER stress protein – distribution

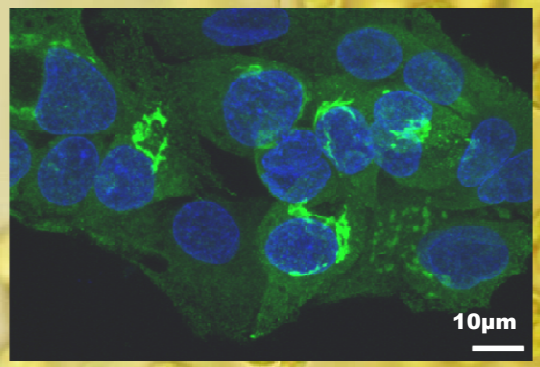
HepG2

Vero

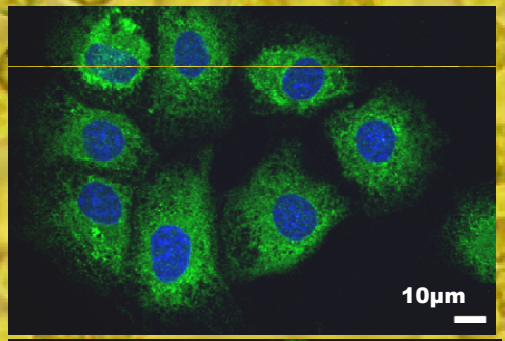


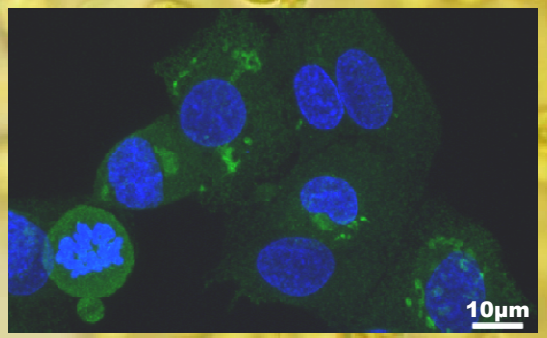
Control



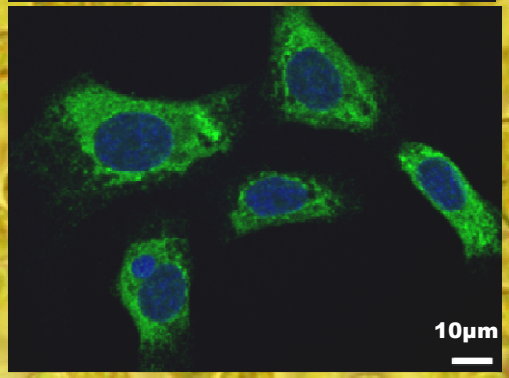


12-50 µM MCLR
Gradual decrease of
green fluorescence





12-50 µM MCLR
Relocalization of GRP94



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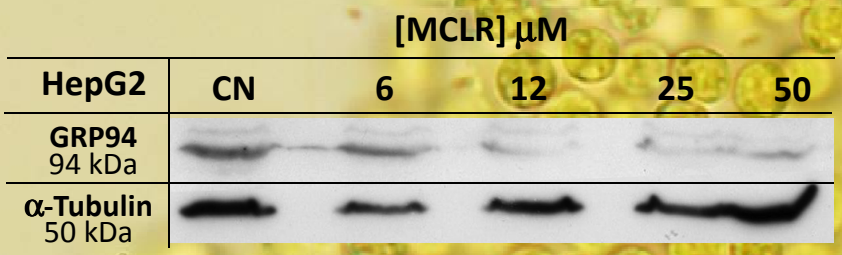
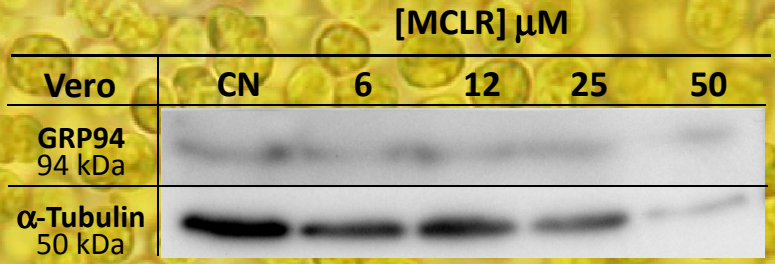
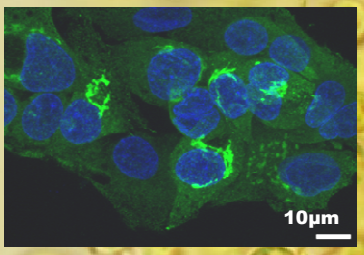
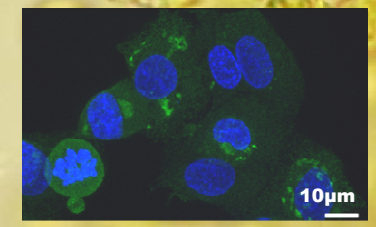
Intracellular targets of MCLR

GRP94 ER stress protein – expression

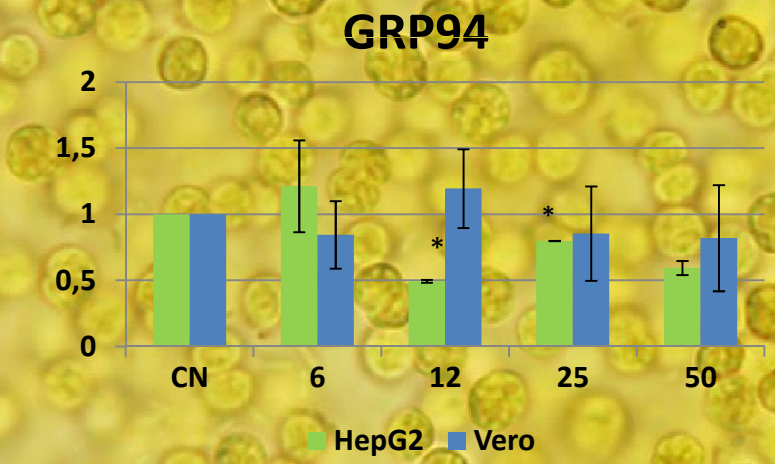


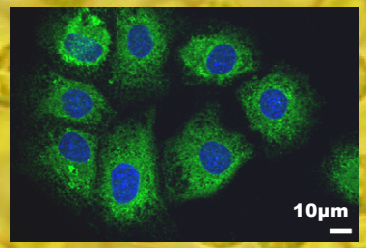


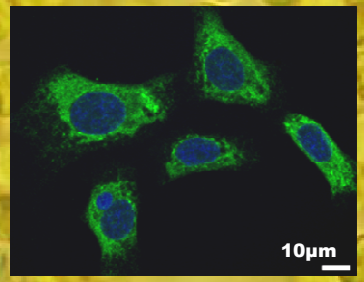




12-50 µM MCLR
Gradual decrease of green fluorescence







12-50 µM MCLR
Relocalization of GRP94

HepG2 cells exposed to MCLR have a decreased expression of GPR94

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Conclusions

MCLR

HepG2
Vero-E6
MDCK
Caco2

Concentration dependent **cell viability** decrease

Autophagolisosomes induction at sub-cytotoxic MCLR concentrations

Concentration dependent **actin** despolimerization

Lysosomes alterations at ↓ concentrations
Mitochondria and **lysosomes** disruption at ↑ concentrations

Endoplasmic reticulum
HepG2: Decrease in the expression of GRP94, autophagy and apoptosis
Vero: Structural alterations and autophagy

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Conclusions

MCLR

HepG2
Vero-E6
MDCK
Caco2

- Concentration dependent cell viability decrease
- Concentration dependent actin despolimerization
- Lysosomes alterations at ↓ concentrations
  Mitochondria and lysosomes disruption at ↑ concentrations
- Autophagolisosomes induction at sub-cytotoxic MCLR concentrations
- Endoplasmic reticulum
  HepG2: Decrease in the expression of GRP94, autophagy and apoptosis
  Vero: Structural alterations and autophagy

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