From Algae to Fish: Unravelling the Toxic Effects of Metformin in Freshwater Ecosystems

Marta Rojais [1,2,3]; Sara C. Antunes [2,3]; Sara Rodrigues [4,5]

[1] School of Medicine and Biomedical Sciences, University of Porto, Porto, Portugal;
[2] Interdisciplinary Centre of Marine and Environmental Research (CIMAR/CIIMAR), University of Porto, Matosinhos, Portugal;
[3] Biology Department, Faculty of Sciences, University of Porto, Porto, Portugal;
[4] Centre of Molecular and Environmental Biology (CBMA), School of Sciences, University of Minho, Braga, Portugal;
[5] Institute of Science and Innovation for Biosustainability (IB-S), School of Sciences, University of Minho, Braga, Portugal

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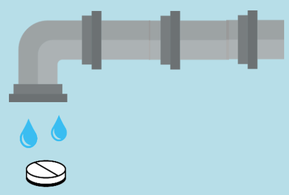
INTRODUCTION

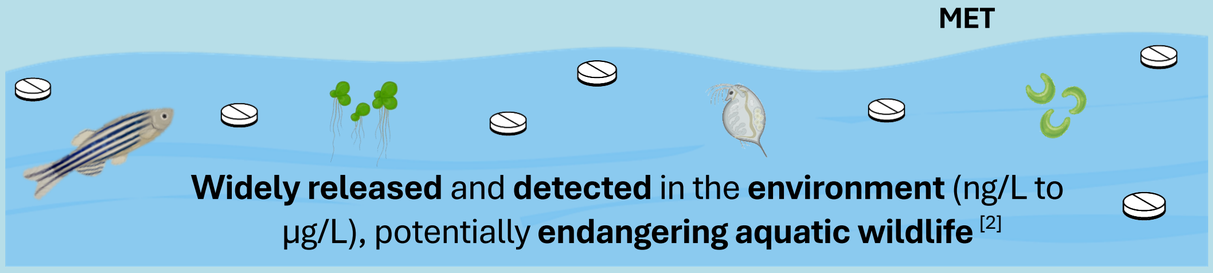
Metformin (MET)

Antidiabetic drug widely prescribed for **Type 2 diabetes mellitus,** with **increasing off-label uses** [1]

Poor removal capacity in **conventional wastewater treatment plants** [2]



MET



Widely released and **detected** in the **environment** (ng/L to µg/L), potentially **endangering aquatic wildlife** [2]

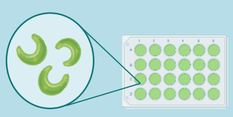
AIM

To evaluate the **short-term ecotoxicological effects** of **MET** on **standard freshwater species**, integrating **physiological responses** and **biochemical markers**

METHODS

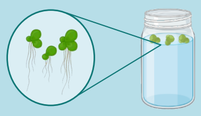
Short- term bioassays

Raphidocelis subcapitata



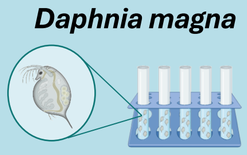
Growth inhibition assay [3]
(3 days; 122.6 – 1000 mg/L)

Lemna minor



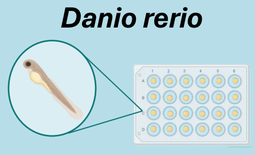
Growth inhibition assay [4]
(7 days; 24.5 – 200 mg/L)

Daphnia magna



Acute immobilization assay [5]
(2 days; 39.2 – 120 mg/L)

Danio rerio



Fish embryo acute toxicity test [6]
(4 days; 0.015 – 3000 mg/L)

Biochemical markers [7]

Oxidative homeostasis
(CAT and GSTs activities; Proline content)

Lipid Peroxidation
(TBARS and MDA levels)

Neurotransmission
(AChE activity)

Photosynthetic pigments
(Total chlorophyll and carotenoids content)

Environmental Hazard Classification

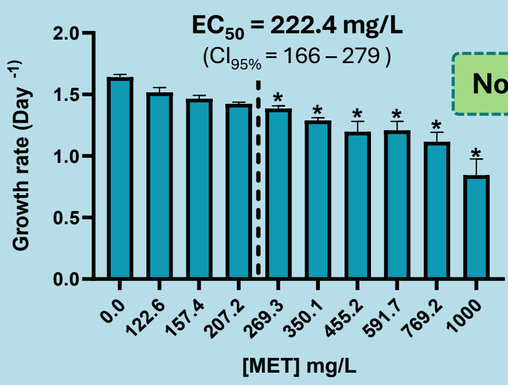
Toxicity score - EU-Directive93/677/ECC [8]

EC_{50} (mg/L)			
> 100	10 - 100	1 - 10	< 1
Non Toxic	Harmful	Toxic	Very toxic

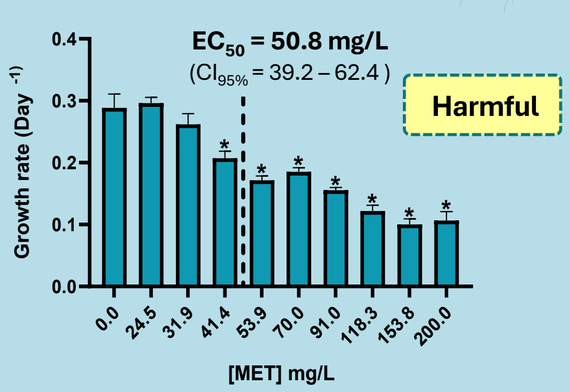
RESULTS & DISCUSSION

Growth Inhibition Assays

R. subcapitata



L. minor



MET significantly inhibited primary producers' growth, with microalgae showing the highest tolerance to MET exposure

D. magna Acute Immobilization Assay

MET (mg/L)	0.00	39.2	51.9	68.6	90.7	120
D	0	0	9	19	29	39
I	0	4	10	7	8	1
ISB	1	1	1	3	3	0
N	39	35	20	11	0	0

***D*- dead; *I*- immobile; *ISB*- irregular swimming behaviour; *N*- normal**

Concentration-dependent increase in immobilisation and mortality effects, with behavioural alterations, preceded mortality

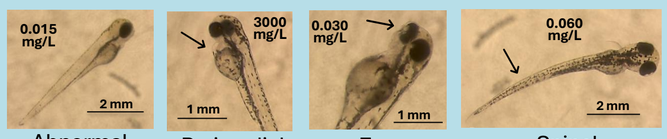
EC_{50} = 53.67 mg/L ($CI_{95\%}$ = 50.0 – 57.3)

Harmful

D. rerio Embryo Acute Toxicity Test

MET did not seem to **affect embryo mortality** or **hatching rates**

Low incidence of developmental abnormalities (< 25%)



Abnormal pigmentation | Pericardial oedema | Eye malformation | Spinal curvature

Biochemical markers

	CAT	GSTs	Proline	MDA or TBARS	AChE	Pigments
L. minor	↗	↗	↗	↗		
D. magna				↗	↘	
D. rerio	↗	↘			↘	

Significant Effect | No Effect | Not evaluated

L. minor
→ **Oxidative damage** in cell membranes
→ **MET did not affect photosynthetic function**

D. magna
→ **MET** induced **oxidative stress** and **neurotoxic responses**

D. rerio
→ **Enhanced antioxidant defences minimizes oxidative damage**
→ **MET** induced **neurotoxicity**

CONCLUSIONS

MET induced adverse effects across **multiple trophic levels**

The **integration** of **life-history traits** with **mechanistic biomarkers** provides a **multilevel perspective** on **MET toxicity**

Chronic effects at **environmentally relevant concentrations** deserve **further investigation**

Urge for **multi-level approaches** in **ecological risk assessment**

References

1. https://doi.org/10.1093/ndt/gfaf177
2. https://doi.org/10.1021/acs.est.2c02495
3. https://doi.org/10.1787/9789264069923-en
4. https://doi.org/10.1787/9789264016194-en
5. https://doi.org/10.1787/9789264069947-en
6. https://doi.org/10.1787/9789264203709-en
7. https://doi.org/10.1007/s11356-024-34659-y
8. https:// op. europa. eu/ en/ publi cation- detai l/-/ publi cation/ 21294 0b8- 3e55- 43f8- 8448- ba258 d0374 bb

Sponsors

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