Effects of metformin and its metabolite guanylurea on fathead minnow (*Pimephales promelas*) reproduction

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Introduction

- Metformin (MET), a medication used to treat type II diabetes, is one of the most prescribed medications worldwide.[1]
- MET is biotransformed to guanylurea (GU) during wastewater treatment.[2]
- Both MET and GU have been reported in surface water at tens to hundreds of µg/L,[2,3] and are frequently detected in Great Lakes tributaries.
- Previous studies have documented an increase in intersex, reduction in fecundity, and alterations in development of teleost fish following exposure to MET or GU in laboratory settings.[4,5] Some studies had a lack of analytical verification or have not been replicated by other laboratories.
- To better characterize their potential ecological effects, we investigated the impacts of MET and GU on reproduction in the fathead minnow (*Pimephales promelas*) using a fish short-term reproduction assay (FSTRA).

Methods

Fish Short-Term Reproduction Assay

- Fathead minnow spawning pairs (n = 12) were exposed for 23 d to one of the following:
  - 0.41, 4.1, 41 µg/L MET
  - 1.0, 10, 100 µg/L GU
  - Control Lake Superior water
- Eggs were counted daily to assess impacts on fecundity.
- Fish were anaesthetized after 23 d and tissues collected.

Chemical Analyses

- MET and GU were confirmed by LC-MS (Agilent 6410 system).
- Replicate tanks (n = 3/treatment) measured at minimum once every 3 days.

Collected tissues:

- Plasma (glucose)
- Liver (RNA)
- Gonad (RNA)

QPCR targets selected to test for impact on reproductive and energy utilization processes:

Gonad:

- *3βHSD,17βHSD,AR,CYP19A1,SULT2A1**

Liver:

- *ESR1,GCK,GYS2,PEPCK,PKLR,SULT2A1,VTG**

Biological Analyses

- RNA extracted using RNeasy Mini (Qiagen).
- QPCRs using SYBR GREEN or TaqMAN RNA-to-Ct kits.

*Gene Targets:

3βHSD	3β-hydroxysteroid dehydrogenase	ESR1	Estrogen Receptor alpha	PEPCK	Phosphoenolpyruvate carboxykinase
17βHSD	17β-hydroxysteroid dehydrogenase	GCK	Glucokinase	PKLR	Pyruvate kinase
AR	Androgen receptor	GYS2	Glycogen synthase 2	SULT2A1	Sulfotransferase 2A1
CYP19A1	Aromatase			VTG	Vitellogenin

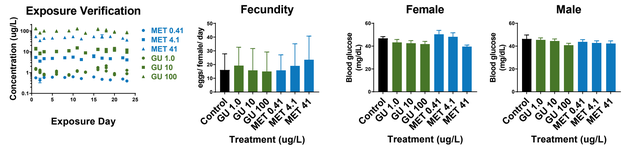
Neither metformin nor guanylurea impacted adult fathead minnow reproduction at environmentally relevant concentrations.

Conclusions and Ongoing Research

- No significant effects were observed after 23 d exposure to environmentally relevant concentrations of MET or GU.
- Though previous studies identified impacts on developing fish, reproduction does not appear to be impacted by MET or GU.
- Ex vivo steroidogenesis assays in progress to assess direct impact of MET or GU on female teleost steroid production.
- Analysis of additional endpoints is ongoing, including metabolomic and transcriptomic profiling of liver tissue. These broader, unsupervised techniques could reveal subtle biological impacts beyond the suite of single gene targets currently presented.

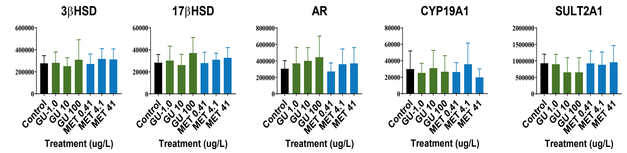
Results

- Chemical exposure was within 25% of nominal for both compounds; No significant differences in fecundity or blood glucose:



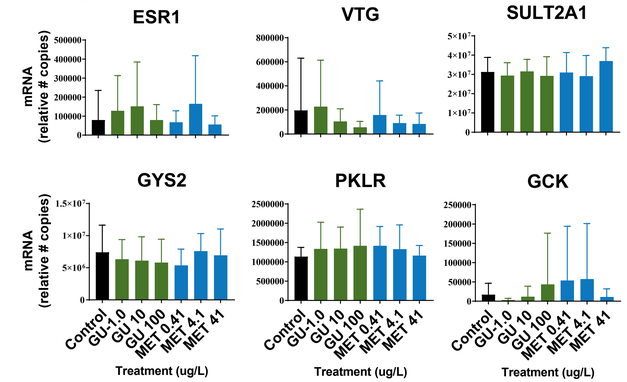
All plots shown as mean ± SD

- No significant differences in transcription of targets in male gonad:



All plots shown as mean ± SD

- No significant differences in transcription of targets in male liver:



All plots shown as mean ± SD

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