Environmental Pollution 254 (2019) 113060

Contents lists available at ScienceDirect

Environmental Pollution

journal homepage: www.elsevier.com/locate/envpol

Cell-based data to predict the toxicity of chemicals to fish. Commentary on the manuscript by Rodrigues et al., 2019. Cell-based assays seem not to accurately predict fish short-term toxicity of pesticides. *Environmental Pollution* 252:476–482[☆,☆☆]

We would like to express strong concerns about the publication by Rodrigues et al., entitled: "Cell-based assays seem not to accurately predict fish short-term toxicity of pesticides", which was recently published in "Environmental Pollution" (2019, 252, pages 476–482, accepted May 07/2019, available online May 27/2019). The topic of the paper is of great interest to the toxicology community because it addresses the need to define alternatives to animals in chemical risk assessment. The authors collected a large amount of in vitro data on chemical testing and added some of their own – the entire data set being predominantly focused on mammalian cell systems - and then attempted a comparison with data for the same chemicals regarding their toxicity to fish. Unfortunately, the work presented is flawed in several ways, sending an undifferentiated, if not wrong, message. Because we fear that this publication can cause unjustified damage to the achievements already made and to the ongoing efforts of the growing community in academia, industry and regulation to further alternatives to animal testing, we wish to openly discuss our concerns.

Inappropriate data analysis

We appreciate the effort by the authors to collect effect data in vivo and in vitro for a wide range of pesticides - we were astonished to find that the authors did not plot the data collected. If they had done so, they would have noticed that their statements regarding the impact of serum content, assay endpoints and exposure time on chemical toxicity are contradictory to their collected data. This can be assessed in the supplemental file that we provide.

Moreover, the authors used the data improperly. As an example, fish LC50s for thiamethoxam were undefined, i.e. the highest concentrations used in the experiments did not cause enough toxicity to determine the LC50, and thus one does not know what the true LC50 is; these values were presented as: >111, >120, >114, >125, >100 mg/L. Still, the authors calculated the geometric mean of these LC50s (i.e. >114 mg/L) which they then divided by the EC50s determined for different cell lines. Not only is this approach inappropriate but, on top of this, all the ratios for thiamethoxam were calculated incorrectly (e.g. LC50/EC50: 114/315 = 0.36 and not 0.036), diminishing the ratios, and thus the apparent sensitivity by the cell lines, by tenfold.

Disregard of almost all prior systematic analyses and misleading referencing

When reading the article, one gets the impression that this is the first report to dig into comparing vertebrate cell-based effect data (mammals, fish) with effect data from fish. This is ignoring a large body of evidence spanning the past ~30 years. To provide just one example, already in 1991, Saito et al. published a study that had all the key words, the authors needed, even in the title: "In vitro cytotoxicity of 45 pesticides to goldfish Gf-scale (Gfs) cells" (Chemosphere 1991, 23, 525–537) - this study, along with a plethora of others, did not make it into the presented analysis; we suggest to the reader to consult the cited Schirmer 2006 review and Kramer et al., 2009 for extensive literature representation of the more historical data. As well, the authors make it sound as if prior work with fish cells concluded that fish cells can rank chemicals in relative terms but are less sensitive than fish with regard to absolute terms. Such findings were indeed reported in early pioneering work decades ago, e.g. by authors such as Saito et al., 1991 and the cited Castaño et al., 1996 or Segner 2004. These findings, hypotheses for their cause, and proposals to overcome such apparent limitations are explored in the review by Schirmer (2006) but not at all taken into account by Rodrigues et al. In the same vein, the authors missed, or ignored, the developments that followed - i.e. consideration of cell line selection, a specifically designed exposure medium and dosing procedure, and accounting for bioavailability by quantification of chemical exposure concentrations in the in vitro systems. The successful implementation of these developments are highlighted in the cited Tanneberger et al., 2013 employing the rainbow trout gill cell line, RTgill-W1, but what Rodrigues et al. withhold from the reader is that the large majority of chemicals tested there, including many pesticides, yielded in vitro effect concentrations that were directly comparable to effect data reported for fish. Instead, they cite the Tanneberger et al., 2013 study solely for the limitations that were discussed there - namely, that the simple cell assay employed was unable to detect the toxicity of neurotoxic chemicals acting through specific channels, such as lindane and permethrin. We would also like to point out that, meanwhile, the RTgill-W1 assay was further tested in an international round-robin study (Fischer et al., 2019, Toxicological Sciences 169 (2), 353–364; Advance Access Publication Date: March 2, 2019) and, though the authors may not have known this at the time of acceptance of their paper on May 07, has in April 2019 been adopted by

DOI of original article: https://doi.org/10.1016/j.envpol.2019.05.033.

☆ This paper has been recommended for acceptance Christian Sonne.

☆☆ Response to this commentary found at: https://doi.org/10.1016/j.envpol.2019.113059.

https://doi.org/10.1016/j.envpol.2019.113060

ISO (ISO21115).

There were other places where research by others is cited in a distorted way. Examples are:

1) Natsch et al. (2018), who tested the RTgill-W1 cell line assay with fragrances, is cited in a way as if they explored the sensitivity of the cell line vs. fish acute toxicity in relative terms although they clearly show the very strong agreement between in vitro and in vivo in absolute terms.
2) The references cited here: "In general, serum-free assays decrease the bioavailability of test compounds in cell-based assays (Al-Sarar et al., 2015a,b; Bertheussen et al., 1997; Ruiz et al., 2006)." actually state that serum-free assays increase the chemical bioavailability and not decrease it.
3) The authors cite the Tanneberger et al. study: "For example, the Tanneberger et al. (2013) study points out that lindane and permethrin (neurotoxic modes of action) were not reflected in the RTgill-W1 assay tested, but the present study found two successful cell-based assays for permethrin (Table S3) …"; however, Rodrigues et al. did not point out that the permethrin EC50 values provided by Tanneberger et al. (between 3.76 and 11.4 mg/L depending if measured or nominal concentrations were used) were up to one order of magnitude lower than the EC50s determined with the "two successful cell-based assays" mentioned by the authors (17 and 49 mg/L). This difference went unnoticed because the permethrin LC50 value taken by Rodrigues et al. was with 5.1 mg/L more than two orders of magnitude higher than that presented by Tanneberger et al., 2013 (0.02 mg/L according to the USEPA fathead minnow data base).

In conclusion, we are highly concerned to see such an improperly executed study. We regret the authors fell short of doing a proper data collection and rigorous meta-analysis. While we agree that enlarging the dataset of adverse in vitro effect concentrations for a wide array of chemicals with varying physico-chemical properties and modes of action, including pesticides, is highly desirable – such endeavors need to include strict data filters that acknowledge the historical and current state of the science. This is something we co-authors value and know is required for advancing methods that will achieve our common goal: replace or reduce the need for animals in chemical risk assessment.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envpol.2019.113060.

Kristin Schirmer*
Eawag, Swiss Federal Institute of Aquatic Science and Technology, 8600 Dübendorf, Überlandstrasse 133, Switzerland

EPF Lausanne, School of Architecture, Civil and Environmental Engineering, 1015 Lausanne, Switzerland

ETH Zürich, Institute of Biogeochemistry and Pollutant Dynamics, 8093 Zürich, Switzerland

Julita Stadnicka-Michalak
Eawag, Swiss Federal Institute of Aquatic Science and Technology, 8600 Dübendorf, Überlandstrasse 133, Switzerland

University of Bern, P.O. Box, 3012 Bern, Switzerland

Scott E. Belanger
The Procter & Gamble Company, Global Product Stewardship, Mason, OH 45040, USA

Ludek Blaha
RECETOX, Masaryk University, Kamenice 5, CZ62500 Brno, Czech Republic

Niels C. Bols
University of Waterloo, 200 University Ave. W, Waterloo, Ontario, Canada N2L 3G1

Scott D. Dyer
LeTourneau University, 2100 S Mobberly Ave, Longview, TX 75602, USA

Michelle R. Embry
Health and Environmental Sciences Institute, 740 15th Street, NW, Suite 600 Washington, DC 20005, USA

Melanie Fischer
Eawag, Swiss Federal Institute of Aquatic Science and Technology, 8600 Dübendorf, Überlandstrasse 133, Switzerland

Marlies Halder[1]
European Commission Joint Research Centre, via E. Fermi 2749, 21027 Ispra VA, Italy

Joop Hermens
Institute for Risk Assessment Sciences, Utrecht University, P.O. Box 80177, 3508 TD Utrecht, the Netherlands

Maria T. Hultman
Norwegian Institute for Water Research, Gaustadallèen 21, 0349 Oslo, Norway

Nynke Kramer
Institute for Risk Assessment Sciences, Utrecht University, P.O. Box 80177, 3508 TD Utrecht, the Netherlands

Heike Laue
In Vitro Molecular Screening, Givaudan Schweiz AG, 8310 Kemptthal, Switzerland

Lucy EJ. Lee
Faculty of Science, University of the Fraser Valley, 33844 King Rd, Abbotsford, BC V2S 7M8, Canada

Adam Lillicrap
Norwegian Institute for Water Research, Gaustadallèen 21, 0349 Oslo, Norway

Andreas Natsch
In Vitro Molecular Screening, Givaudan Schweiz AG, 8310 Kemptthal, Switzerland

Helmut Segner
University of Bern, P.O. Box, 3012 Bern, Switzerland

Katrin Tanneberger
Ecosens AG, 8304 Wallisellen, Grindelstrasse 5, Switzerland

Knut Erik Tollefsen
Norwegian Institute for Water Research, Gaustadallèen 21, 0349 Oslo, Norway

Inge Werner
Swiss Centre for Applied Ecotoxicology, 8600 Dübendorf, Überlandstrasse 133, Switzerland

Hilda Witters
VITO, Flemish Institute for Technological Research, Boeretang 200, 2400 Mol, Belgium

Anze Zupanic
Eawag, Swiss Federal Institute of Aquatic Science and Technology, 8600 Dübendorf, Überlandstrasse 133, Switzerland

National Institute of Biology, Večna pot 111, Ljubljana, Slovenia

* Corresponding author. Eawag, Swiss Federal Institute of Aquatic Science and Technology, Überlandstrasse 133, 8600 Dübendorf, Switzerland.
E-mail address: kristin.schirmer@eawag.ch (K. Schirmer).

12 July 2019

[1] The contents of this correspondence are the views of the author and do not necessarily represent an official position of the European Commission.