Universiteit Utrecht

Department Population Health Sciences
Institute for Risk Assessment Sciences

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Date: 15 August 2021
Subject: Thesis evaluation for Marta Tikhomirov

To the Council of the Scientific Discipline "Veterinary" of Wrocław University of Environmental and Life Sciences

Re: Thesis of Marta Tikhomirov

The thesis of Marta Tikhomirov documents a series of studies in support of the use of intravenous lipid emulsion (ILE) to treat acute opioid poisoning. The candidate approached the question systematically, beginning with the development of an analytical method to measure fentanyl (FEN), buprenorphine (BUP) and butorphanol (BUT) concentrations in cell lysates and rabbit plasma. This first step was essential to be able to conduct the subsequent *in vitro* and *in vivo* studies.

The candidate went on to conduct a series of *in vitro* studies to characterize the partitioning of FEN, BUP and BUT between plasma, plasma proteins, lipids and cell membranes. Next, *in vivo* pharmacokinetic-pharmacodynamic studies showed that ILE alters the pharmacokinetics of both BUP and FEN. However, significant beneficial effects on pharmacodynamic endpoints could only be demonstrated for BUP. Importantly, the administration of ILE did not worsen any pharmacodynamic endpoints for FEN or BUP, and it did not interfere with the antidote nalorphine. It could therefore be concluded that ILE administration is safe and potentially beneficial, especially for BUP overdoses.

This type of data are essential for the rational, safe and effective treatment of humans and animals. The candidate did original work that will make an important, novel contribution to the scientific body of knowledge about the treatment of acute intoxications.

The thesis is well-organized and carefully edited, making it easy to follow and a pleasure to read. The candidate approached the central research question, whether ILE administration would be an effective treatment for acute opioid overdose, systematically. She used a combination of *in vitro* and *in vivo* approaches to gain a full understanding of the system being studied. Her studies were carefully designed with the necessary power calculations, controls and repetitions. Measurement methods were validated and shown to be repeatable.

The systematic, multi-pronged approach made it possible for the candidate to draw meaningful conclusions, which she interpreted in a clinically relevant way. She reported the results of the various studies clearly, using figures and tables effectively. Her interpretations of the results demonstrate an understanding of the characteristics and limitations of the models that were used and how they may be related to the purported mechanisms and *in vivo* observations.

The candidate has thought carefully about the research question and broken it down into clear hypotheses that could be tested with available research techniques and technologies. A clear description of the methods is followed by a meaningful discussion and appropriate conclusions. As is typical for any good research, new questions were generated that are to be addressed in the future. My only criticism is that it would have been nice if the candidate had considered the of Hammarlund-Udenaes and co-workers (e.g., Loryan I, Hammarlund-Udenaes M and Syvänen S. Handb Exp Pharmacol. 2020) as a model to explain the importance of free drug concentrations in plasma and the brain.

In conclusion, this dissertation is the result of a creative approach to an important research question. The studies were well designed and meticulously executed. The interpretation of the results is appropriate. The candidate has demonstrated her ability to think scientifically and conduct research to produce novel results. The dissertation meets the requirements set out in art. 13 ust. 1 ustawy z dnia 14 marca 2003 r. o stopniach naukowych i tytule naukowym oraz o stopniach i tytule w zakresie sztuki.

Sincerely,

Ronette Gehring BVSc, MMedVet (Pharm), PhD

Professor of Veterinary Pharmacotherapy and Pharmacy