EXPOSURE TO PRISTINE PVC MICROPLASTICS RESULTS IN HIGHER REPROTOXICITY IN COMPARISON TO THEIR PHOTOAGED VERSION IN *CAENORHABDITIS ELEGANS* AND IS RELATED TO EXTRACTABLE ADDITIVES

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INTRODUCTION: Quite a great deal of effort has been made to identify the toxicological impacts of microplastics (MPs). However, knowledge on the toxicity of MPs from real environments is still limited. OBJECTIVE: To investigate the reprotoxicity following exposure to PVC microplastics sized 500 µm – 2 mm, subjected or not to UV irradiation, using *Caenorhabditis elegans*. MATERIAL & METHODS: Pristine MPs were degraded through simulation of the natural weathering of plastics, using an UVC chamber. Samples were also submitted to methanolic extraction to isolate the leachable additives/degradation products (further analyzed LC-MS). Plastic degradation was confirmed through color fading, SEM & FTIR. Following, we exposed MD701 synchronized L4 worms for 24 h at 20 ºC in liquid in 24-well plates contaminated with the different MPs (0; 5; 20 and 50 mg/mL) as pristine or degraded MPs, containing *E. coli* OP50 (OD600=24). Using a reporter strain carrying GFP expressed under the control of the *ced-1* promoter, we observed the frequency of germinal cells under apoptosis inside the gonads (n=30-40) using fluorescence microscopy. Worms were also exposed to pristine and photoaged methanol-extracted MPs. At least 3 independent biological replicates were performed. Data was statistically compared using Two-tailed Mann-Whitney test using $P \leq 0.05$. RESULTS & DISCUSSION: Exposure to pristine PVC MPs results in increased germline apoptosis in comparison to their photoaged versions in *Caenorhabditis elegans*. This indicates that PVC MPs weathering, a process that occurs in the natural environment, reduces the toxicity of PVC MPs *in vivo*. Further, after exposing worms to pristine or UV-aged MPs whose additives had been previously extracted with methanol, we saw a significant drop in the frequency of germ cells under apoptosis. Consequently, the additives are the causative agents of reprotoxicity in this experimental

setting. The differences in the chemical profiles obtained in the methanolic extract can explain the frequencies of germinal apoptosis between pristine and UV-degraded microplastics. The insights provided by this study lay the foundation for understanding the environmental risks posed by microplastic additives and products of degradation of “wild” microplastics. ACKNOWLEDGMENTS: We thank Dr. Daiana Ávila for the donation of strain MD701. This work was supported by FONDECYT 11221007, FONDEQUIP EQM150101 and FONDEQUIP EQM160042.