

Supplementary Materials

***Hydra vulgaris* assay as environmental assessment tool for ecotoxicology in freshwaters: a review**

A. Cera^{A,1}, G. Cesarini^{A,1}, F. Spani^{A,B,C} and M. Scalici^A

^ADepartment of Sciences, University of Rome ‘Roma Tre’, Viale Guglielmo Marconi 446, I-00146 Roma, Italy.

^BDepartmental Faculty of Medicine and Surgery, Università Campus Bio-Medico di Roma, Unit of Diagnostic Imaging, Via Alvaro del Portillo 21, I-00128 Roma, Italy.

^CCorresponding author. Email: federica.spani@uniroma3.it

¹These authors contributed equally to present work.

Table S1. List of articles for toxicological and environmental assessment involving *Hydra vulgaris*

For each article several information has been recorded: chemicals or samples analysed (What is analysed); what is investigated (acute toxicity, chronic toxicity, exposure, teratogenicity); which biomarkers have been observed (Biomarker observed); references for the applied protocol (last column)

Ref. number	Article	What is analysed	What is investigated				Biomarker observed	Available references in M&M for the protocol of the assays
			Acute tox.	Chronic tox.	Exp.	Terat.		
1	Ambrosone <i>et al.</i> 2012	CdTe quantum dots		X			growth rate; adult polyp morphology; regeneration morphology; cellular proliferation; molecular analysis	Bosch and David 1984; modified Wilby 1988; Gavrieli <i>et al.</i> 1992; Livak and Schmittgen 2001
2	Ambrosone <i>et al.</i> 2014	SiO ₂ nanoparticles		X			growth rate; adult polyp morphology; feeding behaviour; regeneration morphology; cellular proliferation; molecular analysis	Bosch and David 1984; Ambrosone <i>et al.</i> 2012; David 1973; Livak and Schmittgen 2001
3	Ambrosone <i>et al.</i> 2017	CdSe/ZnS quantum dots	X				growth rate; adult polyp morphology; regeneration morphology	Johnson <i>et al.</i> 1982; Wilby 1988
4	Brown <i>et al.</i> 2014	Effect of clays for detoxification of mycotoxins		X			adult polyp morphology	modified Wilby 1988
5	Cera <i>et al.</i> 2020	Riverine samples			X		regeneration morphology	modified Wilby 1988
6	Hearon <i>et al.</i> 2020	Effects of clay-based sorbents for detoxification		X			adult polyp morphology	Wilby 1988; Brown <i>et al.</i> 2014
7	Huarachi and Gonzalez 2012	Riverine samples		X			adult polyp morphology	Pachura-Bouchet <i>et al.</i> 2006; Castro <i>et al.</i> 2002
8	Kar and Aditya 2007	CdCl ₂		X			adult polyp morphology	not available (the authors compare the LC50 Probit Analysis and a morphological scoring procedure)
9	Lekamge <i>et al.</i> 2018	Ag nanoparticles		X			adult polyp morphology	Trottier <i>et al.</i> 1997
10	Marchesano <i>et al.</i> 2015	Carbon Nano-Onions		X			adult polyp morphology	modified Wilby 1988
11	Murugadas <i>et al.</i> 2016	Copper oxide nanorod		X			growth rate; adult polyp morphology; feeding behaviour; regeneration morphology;	Trottier <i>et al.</i> 1997; Ambrosone <i>et al.</i> 2012; Ambrosone <i>et al.</i> 2014; Buzgariu <i>et al.</i> 2014;

Ref. number	Article number	What is analysed	What is investigated				Biomarker observed	Available references in M&M for the protocol of the assays
			Acute tox.	Chronic tox.	Exp.	Terat.		
12	Murugadas <i>et al.</i> 2019	Bisphenol A	X				cellular proliferation; molecular analysis adult polyp morphology; feeding behaviour; regeneration morphology	Jantzen <i>et al.</i> 1998; Keston and Brandt 1965; Kovačević <i>et al.</i> 2007; Cikala <i>et al.</i> 1999 Murugadas <i>et al.</i> 2016; Ambrosone <i>et al.</i> 2012 ; Ambrosone <i>et al.</i> 2014;
13	Singh and Nel 2017	Riverine samples	X				adult polyp morphology	Trottier <i>et al.</i> 1997; Holdway, 2005; Arkipchuk <i>et al.</i> 2006; Wilby 1988; Blaise and Kusui 1997; Quinn <i>et al.</i> 2009; Persoone <i>et al.</i> 2003
14	Sundaram <i>et al.</i> 2015	Fluoxetine, paroxetinex and citalopram (serotonin reuptake inhibitors) at different pH	X				adult polyp morphology	not available (the authors monitor the survival rate)
15	Traversetti <i>et al.</i> 2017	Riverine samples			X		regeneration morphology	modified Wilby 1988
16	Wang <i>et al.</i> 2017	Effect of enterosorbents to lower toxicity	X				adult polyp morphology	Brown <i>et al.</i> 2014
17	Wang <i>et al.</i> 2019	Effect of clays to reduce chemical exposures	X				adult polyp morphology	Brown <i>et al.</i> 2014
18	Yamindago <i>et al.</i> 2018	Zinc oxide nanoparticles	X				adult polyp morphology, regeneration morphology, histology; molecular analysis growth rate; adult polyp morphology; feeding behaviour; regeneration morphology; histology; cell proliferation; molecular analysis	not available for morphology (tha authors use the LC50 and Probit Analysis)
19	Zeeshan <i>et al.</i> 2017	Co	X					modified Wilby 1988; Marchesano <i>et al.</i> 2015; Ambrosone <i>et al.</i> 2012; Bosch and David 1984; Ambrosone <i>et al.</i> 2014; Zeeshan <i>et al.</i> 2016; Buzgariu <i>et al.</i> 2014; Buzgariu <i>et al.</i> 2008; Technau <i>et al.</i> 2003

References

- Ambrosone, A., Mattera, L., Marchesano, V., Quarta, A., Susha, S. A., Tino, A., Rogach, L. A., and Tortiglione, C. (2012). Mechanisms underlying toxicity induced by CdTe quantum dots determined in an invertebrate model organism. *Biomaterials* **33**, 1991–2000. [doi:10.1016/j.biomaterials.2011.11.041](https://doi.org/10.1016/j.biomaterials.2011.11.041)
- Ambrosone, A., Scotto di Vettimo, M. R., Malvindi, M. A., Roopin, M., Levy, O., Marchesano, V., Pompa, P. P., Tortiglione, C., and Tino, A. (2014). Impact of amorphous SiO₂ nanoparticles on a living organism: morphological, behavioral, and molecular biology implications. *Frontiers in Bioengineering and Biotechnology* **2**(37), 1–12. [doi:10.3389/fbioe.2014.00037](https://doi.org/10.3389/fbioe.2014.00037)
- Ambrosone, A., Roopin, M., Pelaz, B., Abdelmonem, A. M., Ackermann, L., and Mattera, L. (2017). Dissecting common and divergent molecular pathways elicited by CdSe/ZnS quantum dots in freshwater and marine sentinel invertebrates. *Nanotoxicology* **11**(2), 289–303. [doi:10.1080/17435390.2017.1295111](https://doi.org/10.1080/17435390.2017.1295111)
- Arkhipchuk, V. V., Blaise, C., and Malinovskaya, M. V. (2006). Use of hydra for chronic toxicity assessment of waters intended for human consumption. *Environmental Pollution* **142**, 200–211. [doi:10.1016/j.envpol.2005.10.012](https://doi.org/10.1016/j.envpol.2005.10.012)
- Blaise, C., and Kusui, T. (1997). Acute toxicity assessment of industrial effluents with a microplate-based *Hydra attenuata* assay. *Environmental Toxicology and Water Quality* **12**, 53–60. [doi:10.1002/\(SICI\)1098-2256\(1997\)12:1<53::AID-TOX8>3.0.CO;2-7](https://doi.org/10.1002/(SICI)1098-2256(1997)12:1<53::AID-TOX8>3.0.CO;2-7)
- Bosch, T. C., and David, C. N. (1984). Growth regulation in *Hydra*: relationship between epithelial cell cycle lenght and growth rate. *Developmental Biology* **104**, 161–171. [doi:10.1016/0012-1606\(84\)90045-9](https://doi.org/10.1016/0012-1606(84)90045-9)
- Brown, K. A., Mays, T., Romoser, A., Marroquin-Cardona, A., Mitchell, N. J., Elmore, S. E., and Phillips, T. D. (2014). Modified *Hydra* bioassay to evaluate the toxicity of multiple mycotoxins and predict the detoxification efficacy of a clay-based sorbent. *Journal of Applied Toxicology* **34**, 40–48. [doi:10.1002/jat.2824](https://doi.org/10.1002/jat.2824)
- Buzgariu, W., Chera, S., and Galliot, B. (2008). Methods to investigate autophagy during starvation and regeneration in *Hydra*. *Methods in Enzymology* **451**, 409–437. [doi:10.1016/S0076-6879\(08\)03226-6](https://doi.org/10.1016/S0076-6879(08)03226-6)
- Buzgariu, W., Crescenzi, M., and Galliot, B. (2014). Robust G2 pausing of adult stem cells in *Hydra*. *Differentiation* **87**, 83–99. [doi:10.1016/j.diff.2014.03.001](https://doi.org/10.1016/j.diff.2014.03.001)
- Castro, S., Espinola, J., Miguez, D., and Viana, F. (2002) Los bioensayos como herramienta de evaluación de la toxicidad de los efluentes industriales en Uruguay (File 04464); Informe final, International Development Research Centre (IDRC), Montevideo, Uruguay.
- Cera, A., Ceschin, S., Del Grosso, F., Traversetti, L., and Scalici, M. (2020). Correlating ecotoxicological early-warning systems to biotic indices to assess riverine teratogenic contamination. *Marine and Freshwater Research* **71**(8), 1033–1039. [doi:10.1071/MF18471](https://doi.org/10.1071/MF18471)
- Cikala, M., Wilm, B., Hobmayer, E., Bottger, A., and David, C. N. (1999). Identification of caspases and apoptosis in the simple metazoan *Hydra*. *Current Biology* **9**, 959–962. [doi:10.1016/S0960-9822\(99\)80423-0](https://doi.org/10.1016/S0960-9822(99)80423-0)
- David, C. (1973). A quantitative method for maceration of hydra tissue. *Wilhelm Roux' Archiv für Entwicklungsmechanik der Organismen* **171**, 259–268. [doi:10.1007/BF00577724](https://doi.org/10.1007/BF00577724).

- Gavrieli, Y., Sherman, Y., and Ben-Sasson, S. A. (1992). Identification of programmed cell death in situ via specific labeling of nuclear DNA fragmentation. *The Journal of Cell Biology* **119**, 493–501. [doi:10.1083/jcb.119.3.493](https://doi.org/10.1083/jcb.119.3.493)
- Hearon, S. E., Wang, M., and Phillips, T. D. (2020). Strong adsorption of dieldrin by parent and processed montmorillonite clays. *Environmental Toxicology and Chemistry* **39**(3), 517–525. [doi:10.1002/etc.4642](https://doi.org/10.1002/etc.4642)
- Holdway, D. A. (2005). Hydra population reproduction toxicity test method. In ‘Small-scale Freshwater Toxicity Investigations’. (Eds C. Blaise, and J.-F. Féral.) Vol. 1, pp. 395–411. (Springer Netherlands.)
- Huarachi, R., and Gonzalez, R. (2012). *Hydra vulgaris* Pallas, 1766 (Hydrozoa: Hydriidae) as bioindicator of the water quality of the river Chili, Arequipa, Peru. *The Biologist* **10**, 125–137.
- Jantzen, H., Hassel, M., and Schulze, I. (1998). Hydroperoxides mediate lithium effects on regeneration in *Hydra*. *Comparative Biochemistry and Physiology – C. Toxicology & Pharmacology* **119**, 165–175. [doi:10.1016/S0742-8413\(97\)00204-1](https://doi.org/10.1016/S0742-8413(97)00204-1)
- Johnson, E. M., Gorman, R. M., Gabel, B. E., and George, M. E. (1982). The *Hydra attenuata* system for detection of teratogenic hazards. *Teratogenesis, Carcinogenesis, and Mutagenesis* **2**, 263–276. [doi:10.1002/1520-6866\(1990\)2:3/4<263::AID-TCM1770020308>3.0.CO;2-I](https://doi.org/10.1002/1520-6866(1990)2:3/4<263::AID-TCM1770020308>3.0.CO;2-I)
- Kar, S., and Aditya, A. K. (2007). Evaluation of freshwater toxicity with *Hydra* as a test animal. *Philippine Journal of Science* **136**, 173–179.
- Keston, A. S., and Brandt, R. (1965). The fluorometric analysis of ultramicro quantities of hydrogen peroxide. *Analytical Biochemistry* **11**, 1–5. [doi:10.1016/0003-2697\(65\)90034-5](https://doi.org/10.1016/0003-2697(65)90034-5)
- Kovačević, G., Želježić, D., Horvatin, K., and Kalafatić, M. (2007). Morphological features and comet assay of green and brown hydra treated with aluminium. *Symbiosis* **44**, 145–152.
- Lekamge, S., Miranda, A. F., Abraham, A., Li, V., Shukla, R., Bansal, V., and Nugegoda, D. (2018). The toxicity of silver nanoparticles (AgNPs) to three freshwater invertebrates with different life strategies: *Hydra vulgaris*, *Daphnia carinata*, and *Paratya australiensis*. *Environmental Sciences* **6**, 152. [doi:10.3389/fenvs.2018.00152](https://doi.org/10.3389/fenvs.2018.00152)
- Livak, K. J., and Schmittgen, T. D. (2001). Analysis of relative gene expression data using realtime quantitative PCR and the $2^{-\Delta\Delta CT}$ method. *Methods* **25**, 402–408. [doi:10.1006/meth.2001.1262](https://doi.org/10.1006/meth.2001.1262)
- Marchesano, V., Ambrosone, A., Bartelmess, J., Strisciante, F., Tino, A., Echegoyen, L., Tortiglione, C., and Giordani, S. (2015). Impact of carbon nano-onions on *Hydra vulgaris* as a model organism for nanotoxicology. *Nanomaterials* **5**, 1331–1350. [doi:10.3390/nano5031331](https://doi.org/10.3390/nano5031331)
- Murugadas, A., Zeeshan, M., Thamaraiselvi, K., Ghaskadbi, S., and Akbarsha, M. A. (2016). *Hydra* as a model organism to decipher the toxic effects of copper oxide nanorod: eco-toxicogenomics approach. *Scientific Reports* **6**, 29663. [doi:10.1038/srep29663](https://doi.org/10.1038/srep29663)
- Murugadas, A., Mahamuni, D., Nirmaladevi, S. D., Thamaraiselvi, K., Thirumurugan, R., and Akbarsha, M. A. (2019). *Hydra* as an alternative model organism for toxicity testing: study using the endocrine disrupting chimica bisphenol A. *Biocatalysis and Agricultural Biotechnology* **17**, 680–684. [doi:10.1016/j.biab.2019.01.009](https://doi.org/10.1016/j.biab.2019.01.009)
- Pachura-Bouchet, S., Blaise, C., and Vasseur, P. (2006). Toxicity of nonylphenol on the cnidarian *Hydra attenuata* and environmental risk assessment. *Environmental Toxicology* **21**, 388–394. [doi:10.1002/tox.20201](https://doi.org/10.1002/tox.20201)

- Persoone, G., Marsalek, B., Blinova, I., Torokne, A., Zarina, D., Manusadzianas, L., Nalecz-Jawecki, G., Tofan, L., Stepanova, N., Tothova, L., and Kolar, B. (2003). A practical and user-friendly toxicity classification system with microbiotests for natural and wastewaters. *Environmental Toxicology* **18**(6), 395–402. [doi:10.1002/tox.10141](https://doi.org/10.1002/tox.10141)
- Quinn, B., Gagné, F., and Blaise, C. (2009). Evaluation of the acute, chronic and teratogenic effects of a mixture of eleven pharmaceuticals on the cnidarian, *Hydra attenuata*. *The Science of the Total Environment* **407**, 1072–1079. [doi:10.1016/j.scitotenv.2008.10.022](https://doi.org/10.1016/j.scitotenv.2008.10.022)
- Singh, P., and Nel, A. (2017). A comparison between *Daphnia pulex* and *Hydra vulgaris* as possible test organisms for agricultural run-off and acid mine drainage toxicity assessments. *Water S.A.* **43**, 323–332. [doi:10.4314/wsa.v43i2.15](https://doi.org/10.4314/wsa.v43i2.15)
- Sundaram, R., Smith, B. W., and Clark, T. M. (2015). PH-dependent toxicity of serotonin selective reuptake inhibitors in taxonomically diverse freshwater invertebrate species. *Marine and Freshwater Research* **66**, 518–525. [doi:10.1071/MF14015](https://doi.org/10.1071/MF14015)
- Technau, U., Miller, M. A., Bridge, D., and Steele, R. E. (2003). Arrested apoptosis of nurse cells during *Hydra* oogenesis and embryogenesis. *Developmental Biology* **260**, 191–206. [doi:10.1016/S0012-1606\(03\)00241-0](https://doi.org/10.1016/S0012-1606(03)00241-0)
- Traversetti, L., Del Gross, F., Malafoglia, V., Colasanti, M., Ceschin, S., Larsen, S., and Scalici, M. (2017). The *Hydra* regeneration assay reveals ecological risks in running waters: a new proposal to detect environmental teratogenic threats. *Ecotoxicology* **26**, 184–195. [doi:10.1007/s10646-016-1753-4](https://doi.org/10.1007/s10646-016-1753-4)
- Trottier, S., Blaise, C., Kusui, T., and Johnson, E. M. (1997). Acute toxicity assessment of aqueous samples using a microplate-based *Hydra attenuata* assay. *Environmental Toxicology and Water Quality* **12**, 265–271. [doi:10.1002/\(SICI\)1098-2256\(1997\)12:3<265::AID-TOX10>3.0.CO;2-9](https://doi.org/10.1002/(SICI)1098-2256(1997)12:3<265::AID-TOX10>3.0.CO;2-9)
- Wang, M., Maki, C. R., Deng, Y., Tian, Y., and Phillips, T. D. (2017). Development of high capacity enterosorbents for aflatoxin B1 and other hazardous chemicals. *Chemical Research in Toxicology* **30**, 1694–1701. [doi:10.1021/acs.chemrestox.7b00154](https://doi.org/10.1021/acs.chemrestox.7b00154)
- Wang, M., Hearon, S. E., Johnson, N. M., and Phillips, T. D. (2019). Development of broad-acting clays for the tight adsorption of benzoapyrene and aldicarb. *Applied Clay Science* **168**, 196–202. [doi:10.1016/j.clay.2018.11.010](https://doi.org/10.1016/j.clay.2018.11.010)
- Wilby, O. K. (1988). The *Hydra* regeneration assay. In ‘Proceedings of Workshop Organized by Association Francaise de Teratologie’, 3 June 1988, Royaumont, France. pp. 108–124. (Association Francaise de Teratologie.)
- Yamindago, A., Lee, N., Woo, S., Choi, H., Young Mun, J., Jang, S. W., Yang, S. I., Erxleben, F. A., Bosch, T. C. G., and Yum, S. (2018). Acute toxic effects of zinc oxide nanoparticles on *Hydra magnipapillata*. *Aquatic Toxicology* **205**, 130–139. [doi:10.1016/j.aquatox.2018.10.008](https://doi.org/10.1016/j.aquatox.2018.10.008)
- Zeeshan, M., Murugadas, A., Ghaskadbi, S., Rajendran, R. B., and Akbarsha, M. A. (2016). ROS dependent copper toxicity in *Hydra*- Biochemical and molecular study. *Comparative Biochemistry and Physiology. Toxicology & Pharmacology : CBP* **185–186**, 1–12. [doi:10.1016/j.cbpc.2016.02.008](https://doi.org/10.1016/j.cbpc.2016.02.008)
- Zeeshan, M., Murugadas, A., Ghaskadbi, S., Ramaswamy, B. R., and Akbarsha, M. A. (2017). Ecotoxicological assessment of cobalt using *Hydra* model: ROS, oxidative stress, DNA damage, cell cycle arrest, and apoptosis as mechanisms of toxicity. *Environmental Pollution* **224**, 54–69. [doi:10.1016/j.envpol.2016.12.042](https://doi.org/10.1016/j.envpol.2016.12.042)