Environmental Toxicology

Overview:

C. elegans is a well-validated experimental subject for toxicological research and recommended by NIH for whole-animal toxicology and safety testing (1, 2). High-throughput toxicology screening systems are available for *C. elegans*, using endpoints such as locomotion or development. The ScreenChip system provides lower throughput but greater experimental power to investigate underlying mechanisms, especially for neurotoxins. By providing a direct, electrical readout of a worm's physiological health, the ScreenChip System offers a real-time readout of toxic effects. Electropharyngeogram (EPG) recordings can be combined with molecular-genetic manipulations (e.g., of genes that influence sensitivity or resistance to toxins) and simultaneous imaging using one or more multiple fluorescent markers, from L1 to the adult stage. This state-of-the-art technology provides exceptional ease-of-use and statistical power to:

- Investigate molecular, cellular and organ-level responses to environmental toxins.
- Obtain a direct readout of ion channels and neurotransmitter receptor activity that may be disrupted by neurotoxins.
- Identify genes and gene networks involved in defenses against toxic substances or that enhance susceptibility.
- Study differences between acute vs. chronic exposure, synergistic effects, etc.
- Rapidly detect environmental toxins, faster than other whole-animal assays.
- Compare toxic effects during the *C. elegans* life cycle from L1 to adult.
- Simultaneously image fluorescent reporters while recording EPGs
- Investigate other adverse stimuli such as oxidative stress or hypoxia.

Heavy metals:

Copper (Cu 2+)

Exposure to elevated levels of Cu 2+, e.g., from corroded plumbing systems, is toxic to humans. Copper pollution also threatens aquatic wildlife such as salmon.

Using the ScreenChip System, we confirmed the toxicity of Cu 2+ in *C. elegans* (3), indicated by the concentration-dependent inhibition of pumping after a 60-min exposure (Fig. 1). At the higher Cu 2+ concentrations, pumping ceases within 5 min (data not shown), providing dramatically faster detection of toxicity than standard whole-animal assays [e.g., growth and reproduction in *C. elegans*, fish lethality]. The calculated IC 50 (concentration at which pump frequency was reduced by 50%) of Cu 2+ is within the range found in contaminated tap water.

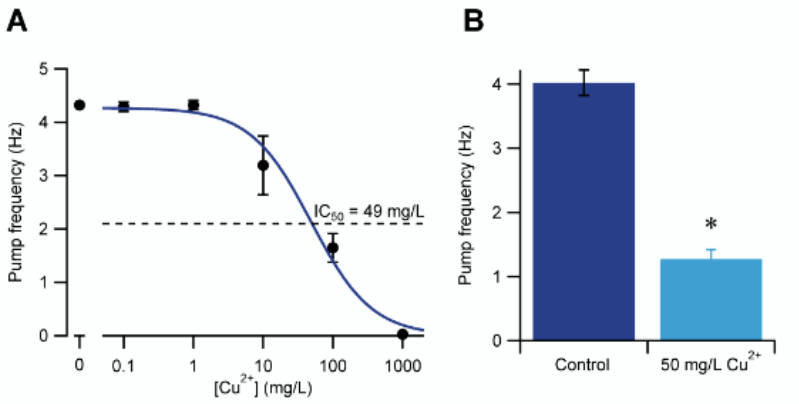

Fig 1. Copper toxicity. (A): Day 1 adult N2 worms were cultured in medium with Cu^{2+} or solvent. After 60 min, 8-channel EPG chip recordings (4) were made in 10 mM 5HT in M9 for 60 min. Steady-state pump frequency was plotted using the Hill equation (*n* = 11-15 worms/group; lines and shading show mean ± S.E.M.); (B): ScreenChip System recordings (2-3 min in duration) were begun 30 min after the onset of exposure to 50 mg/L Cu^{2+} or solvent (*n* = 26-28 worms/group; mean ± S.E.M.). The Cu^{2+}-exposed group showed a significant reduction in pump frequency compared to controls (*, $P < 10^{-5}$; 2-tailed Mann-Whitney Wilcoxon Test).

Cadmium (Cd 2+)

Routes of human exposure to Cd 2+ include fossil fuel combustion, municipal waste incineration, fertilizers, tobacco smoking, contaminated food, and industrial soil and water pollution. Cd 2+ contributes to cardiovascular and kidney disease and threatens wildlife. As done above with Cu 2+ , we confirmed that Cd 2+ is toxic to *C. elegans* (3), indicated by the concentration-dependent inhibition of pumping following a 60 min exposure (Fig. 2). At higher concentrations, inhibition occurred within 10 min (data not shown), again illustrating the rapidity with which the ScreenChip System can provide toxicity data. Cadmium causes rapid electrophysiological effects (e.g., blockade of Ca 2+ channels in heart muscle) as well as chronic effects that accumulates over time. The IC 50 for Cd 2+ is approximately double the [Cd 2+] found in water contaminated by industrial pollution; samples can be brought into the range of the Cd 2+ dose-response curve by providing a longer exposure or concentrating the samples.

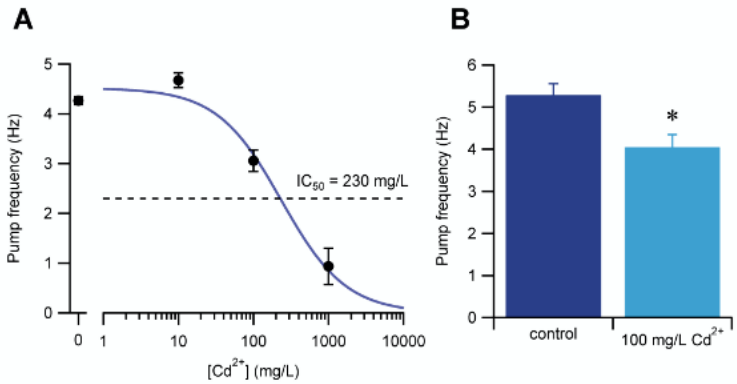

Fig. 2. Cadmium toxicity. Same methods as in Fig. 1. (A): Steady-state pump frequency was plotted against Cd 2+ concentration using the Hill equation. n = 11-15 worms/group. (B): ScreenChip System recordings (2 min) were begun 30 min after the onset of exposure to 100 mg/L Cd 2+ or solvent (n = 21-

27 worms/group). The Cd 2+ -exposed group showed a significant reduction in pump frequency compared to controls (*, P =0.002; 2-tailed Mann-Whitney Wilcoxon Test).

Dichlorvos – an organophosphate insecticide:

Like other organophosphates, dichlorvos is a neurotoxin that inhibits acetylcholinesterase, an enzyme involved in synaptic transmission. Environmental contamination results primarily from agricultural uses and aerial spraying; the chemical is banned in Europe. Health risks from chronic exposure include neurological and cognitive dysfunction, including increased risk for ADHD in children. Dichlorvos is also toxic to honeybees, fish, birds and other wildlife. A recent survey of U.S. rivers and streams showed that almost half had dichlorvos levels over the benchmark for aquatic health (5).

As seen in Fig. 3, the ScreenChip System confirms the toxicity of dichlorvos on *C. elegans* (6), manifested by a concentration-dependent inhibition of pharyngeal pumping after a 24 h incubation in 0 to 80 µM dichlorvos. The World Health Organization considers dichlorvos >0.1 µM in drinking water to be dangerous, a value potentially detectable by the ScreenChip system even without concentrating water samples.

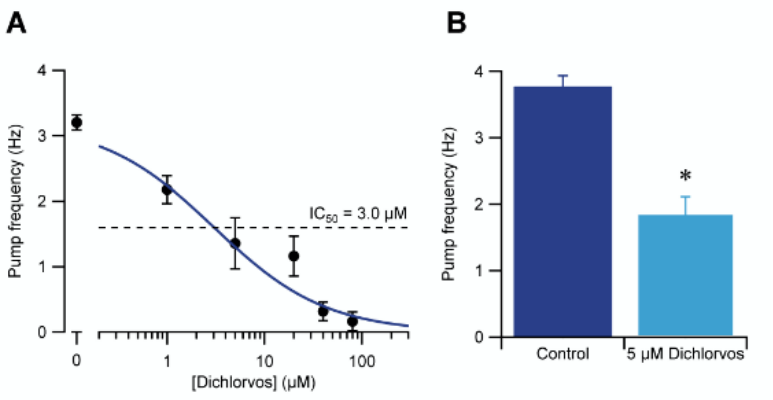

Fig. 3. Dichlorvos toxicity. (A): Same methods as Fig. 1 and 2 except worms were cultured for 24 hrs in medium with *E. coli* and dichlorvos or solvent. Steady-state pump frequency was plotted using the Hill equation. Controls, *n* = 55; dichlorvos, *n* = 7-24 worms/group. (B): ScreenChip System recordings (2-3 min) were taken 24 hrs after the onset of exposure to 5 µM dichlorvos or solvent. Controls, *n* = 64; 5 µM dichlorvos, *n* = 88 worms/group. The dichlorvos-exposed group showed a significant reduction in pump frequency compared to controls (*, $P < 10^{-12}$; 2-tailed Mann-Whitney Wilcoxon Test).

References:

(1) Leung MC et al. (2008) *Caenorhabditis elegans*: an emerging model in biomedical and environmental toxicology. Toxicol Sci. 106(1):5-28; Honnen S (2017) *Caenorhabditis elegans* as a powerful alternative model organism to promote research in genetic toxicology and biomedicine. Arch Toxicol. May;91(5): 2029-2044. doi: 10.1007/s00204-017-1944-7. Epub 2017 Mar 15.

(2) *C. elegans*: a medium-throughput screening tool for toxicology (2006). http://ntp.niehs.nih.gov/ntp/factsheets/wormtoxfs06.pdf

(3) Jiang Y et al. (2016) Sublethal toxicity endpoints of heavy metals to the nematode *Caenorhabditis elegans*. PLoS One, 11(1):e0148014.

(4) Lockery S.R., Hulme S.E., Roberts W.M., Robinson K.J., Laromaine A., Lindsay T.H., Whitesides G.M., Weeks J.C. A microfluidic device for whole-animal drug screening using electrophysiological measures in the nematode *C. elegans*. Lab. Chip. 2012;12:2211–2220. [PubMed]

(5) Stone WW, Gilliom RJ, Ryberg KR (2014) Pesticides in U.S. Streams and Rivers: Occurrence and Trends during 1992–2011 *Environ. Sci. Technol.*, 2014, 48 (19):11025–30

(6) Jadhav KB, PS Rajini (2009) Evaluation of sublethal effects of dichlorvos upon *Caenorhabditis elegans* based on a set of end points of toxicity. J Biochem Molecular Toxicology 23(1): 9-17

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Tech Note: http://nemametrix.com/epg-recordings-new-tool