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A HIGH-THROUGHPUT METHOD FOR ASSESSING CHEMICAL TOXICITY USING A *CAENORHABDITIS ELEGANS* REPRODUCTION ASSAY

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Abstract

The National Research Council has outlined the need for non-mammalian toxicological models to test the potential health effects of a large number of chemicals while also reducing the use of traditional animal models. The nematode *Caenorhabditis elegans* is an attractive alternative model because of its well-characterized and evolutionarily-conserved biology, low cost, and ability to be used in high-throughput screening. A high-throughput method is described for quantifying the reproductive capacity of *C. elegans* exposed to chemicals for 48 h from the last larval stage (L4) to adulthood using a COPAS Biosort. Initially, the effects of exposure conditions that could influence reproduction were defined. Concentrations of DMSO vehicle ≤ 1% did not affect reproduction. Previous studies indicated that *C. elegans* may be influenced by exposure to low pH conditions. At pHs greater than 4.5, *C. elegans* reproduction was not affected, however below this pH there was a significant decrease in the number of offspring. Cadmium chloride was chosen as a model toxicant to verify that automated measurements were comparable to those of traditional observational studies. EC_{50} values for cadmium for automated measurements (176-192 μM) were comparable to those previously reported for a 72-h exposure using manual counting (151 μM). The toxicity of seven test toxicants on *C. elegans* reproduction was highly correlative with rodent lethality suggesting that this assay may be useful in predicting the potential toxicity of chemicals in other organisms.

Keywords

Caenorhabditis elegans; high-throughput screening; alternative toxicological models; cadmium

Introduction

The National Research Council and government agencies including the National Institutes of Health and the U.S. Environmental Protection Agency have defined a need for reliable high-throughput screening (HTS) methods to evaluate the potential human health impacts of the

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large number of chemicals in production (National Research Council, 2000; Dix *et al.*, 2007). Although rodent-based assays have been the traditional model for toxicological studies, many agencies are now recognizing the advantages of *in vivo* whole organism studies using invertebrate species. These advantages include rapid and inexpensive testing, as well as a lack of animal welfare issues. Although *in vitro* cell-based HTS assays are commonly used, whole organism testing allows researchers to observe phenotypes that are well-characterized and biologically relevant.

The nematode *Caenorhabditis elegans*, a popular model organism for genetic and developmental biology research, is now being recognized as an attractive invertebrate model for high-throughput toxicological studies. *C. elegans* has a rapid and well-characterized life cycle and can be cultured in multi-well plates, making them amenable to HTS. There is also a high degree of conservation between *C. elegans* and mammalian species in processes controlling development, neurobiology, and stress responses (Kaletta and Hengartner, 2006). For these reasons, several pharmaceutical companies are using *C. elegans* as part of their drug discovery process (Artal-Sanz *et al.*, 2006).

One easily quantifiable phenotype in *C. elegans* is reproduction. *C. elegans* develop from embryo to gravid adult through four distinct larval stages, termed L1-L4, in about three days at 20°C (Wood, 1988). At the L4 stage, germ cells within *C. elegans* hermaphrodites mature to sperm, while in the adult stage germ cells mature to oocytes. Following internal fertilization, the developing embryos are released by muscle contractions of the vulva, which are regulated by specific neurons that release serotonin, acetylcholine, or neuropeptides (Trent *et al.*, 1983; Weinshenker *et al.*, 1995; Bany *et al.*, 2003).

Reproduction and egg-laying are affected by a number of environmental conditions including salt concentration (Horvitz *et al.*, 1982) and food availability (Trent *et al.*, 1983). Exposure to ethanol (Dhawan *et al.*, 1999), metals (Anderson *et al.*, 2001), Enterobacteriaceae (Sicard *et al.*, 2007), anthelmintic agents (Kim *et al.*, 2001), and nicotinic agonists (Kim *et al.*, 2001; Bull *et al.*, 2007) also affect reproduction. Because such a wide variety of toxicants impact *C. elegans* reproduction, it is a promising endpoint for HTS. Egg-laying and reproduction have been measured in low throughput by placing one to several nematodes on the surface of an agar plate or in liquid media. After exposure, the number of offspring was manually counted with the aid of a microscope. Automated tracking systems and image analysis have also been used to monitor the frequency of egg-laying behavior on agar surfaces over several minutes to hours (Kim *et al.*, 2001; Davies *et al.*, 2003; Geng *et al.*, 2005). A more high-throughput, but indirect approach to quantify reproduction, measures chitinase, which is released during embryo hatching (Kaletta and Hengartner, 2006).

In this report, an automated HTS method is described that directly quantifies the number of *C. elegans* offspring after exposure to potential toxicants. In this assay, a COPAS Biosort (Pulak, 2006) is used to load L4 hermaphrodite nematodes into each well of a 96-well plate containing test chemicals. Following a 48-h incubation, the number of offspring in each well is quantified using the Biosort. In the presence of the test chemicals, there were toxicant and concentration dependent decreases in the level of reproduction. These decreases could be the result of reducing the number of sperm or oocytes, disrupting germ cell maturation, affecting egg-laying behavior, or increasing embryonic or larval lethality after egg-laying. The strength of this assay was evaluated by comparing the toxicity of several different classes of chemicals in the *C. elegans* assay to toxicity measures in mice and rodents.

Methods

Nematode culture

The Bristol N2 (wild-type) and CB5584 *mIs12[myo-2::GFP, pes-10::GFP, F22B7.9::GFP]* (referred to as *myo-2::GFP)* strains of *C. elegans* were obtained from the *Caenorhabditis* Genetic Center (Minneapolis, MN) and maintained at 20°C on K-agar plates (2% bacto-agar, 0.25% bacto-peptone, 51 mM sodium chloride, 32 mM potassium chloride, 13 μM cholesterol) seeded with *E. coli* OP50 (Williams and Dusenbery, 1988). Age-synchronized adult nematodes were prepared as previously described (Khanna *et al.*, 1997).

Reproduction assay

Using a COPAS Biosort (Union Biometrica Inc., Somerville, MA, USA), five L4 nematodes were added to each well of a 96-well plate, containing a final volume of 50 μl K-medium (51 mM NaCl, 32 mM KCl), *E. coli*, and test chemical. Stock solutions of test chemicals were prepared in K-medium or DMSO (final concentration ≤ 1% DMSO; see below) depending on the chemical's aqueous solubility. The bacterial concentration was measured by determining the optical density at 550 nm immediately before nematode addition. Nematodes were incubated for 48 h, after which adults and offspring were aspirated using a Biosort. The Biosort records up to four attributes for each individual nematode: TOF, which relates to nematode length; EXT, which corresponds to the optical density; and two fluorescence measurements. TOF and EXT measurements are related to the age and size of the nematode; both increase as *C. elegans* develop. The TOF, EXT, and level of green fluorescence of individual *C. elegans* were recorded along with the total number of nematodes sampled per well.

Each treatment group consisted of six exposure wells (i.e. total of thirty parents per treatment condition) followed by two rinse wells to minimize carry-over of offspring between treatment groups. Total reproductive counts (i.e., number of non-adult nematodes) were used as the endpoint of the assay. Each experiment was replicated three times. Preliminary experiments were performed on untreated nematodes to determine the optimum number of parents per well and exposure duration to minimize count variability and carry-over between wells (data not shown).

Concentration-response curves

The reproduction assay was used to test the effects of eight chemicals on wild-type and *myo-2::GFP* strains of *C. elegans*. The relationship between chemical concentration and reproductive counts was modeled using a four parameter sigmoidal growth model (Copeland, 2000). The model, also known as the Morgan-Mercer-Flodin model (Ratkowsky, 1983), was parameterized as follows:

$$v = V_A + \frac{V_B [S]^h}{[S]_{50}^h + [S]^h} \quad \{1\}$$

Here, v is taken to be the reproductive count at concentration [S]. V_A and V_B are the reproductive counts at the lower and upper asymptotes of the curve, respectively. $[S]_{50}$ is the effective concentration producing 50% of the maximal reproductive count, which we will refer to as the EC_{50}. h is the Hill coefficient. All parameters, including EC_{50}s and confidence intervals were estimated using likelihood methods assuming constant variance in reproductive counts across concentration levels.

For each experiment, the benchmark concentration (BMC), which is the concentration corresponding to a specified excess risk relative to the control response was calculated as

previously described (Crump, 2002). The BMC method has been suggested as an alternative to the traditional no observable adverse effect level (NOAEL) (Sand *et al.*, 2008). The BMC was defined as the concentration that reduced the reproductive count by 10% relative to the untreated or control group. The control group was assumed to be normally distributed with mean and standard deviation estimated from the likelihood fit to equation {1}, and an adverse effect to be the first percentile of counts in the controls. The corresponding lower limit of a one-sided 95% confidence interval on the BMC was referred to as the BMCL, and was calculated using bootstrap methods. Technical details are provided in Supplemental Table 1.

Effects of pH

Two acids (ascorbic and acetic) were chosen to test the effects of pH on wild-type and *myo-2::GFP* reproduction. The pH was measured using an Orion 911600 semi-micro pH electrode (Thermo Scientific, Beverly, MA, USA) and a Pinnacle series M 540P pH meter (NovaAnalytics Corp, Woburn, MA, USA). After a 48-h exposure at the indicated pH, the number of nematodes was counted using the Biosort.

All calculations of the relationship between pH and reproductive counts were performed as described above for chemical exposures except that *v* in Equation {1} is the reproductive count at pH [S]. The pH producing 50% of the maximal reproductive count is referred to as the pH_{50} and the benchmark pH (BMpH) as the pH that decreased the reproductive count by 10% relative to the control group. Additional details are provided in Supplemental Table 2.

Results

Reproduction of untreated *C. elegans*

In the *C. elegans* reproduction assay, nematodes are exposed to chemicals from their last larval (L4) through the adult stage, and then their offspring are counted using a COPAS Biosort. Two strains of *C. elegans* that are routinely used in toxicological assays in our laboratory were examined: wild-type and *myo-2::GFP*. The *myo-2::GFP* strain, which has a fluorescently-labeled pharynx, is being used in the development of additional toxicological HTS assays, including motion tracking assays and assays in which discriminating the anterior-posterior orientation is required (Boyd *et al.*, 2010).

At the beginning of each experiment, five L4 animals were loaded into each of six wells of a 96-well plate (i.e., 30 L4s total per treatment group). The average number of offspring recovered after 48 h for untreated animals ranged from 33 - 43 per parent. Figure 1 presents a typical distribution of an untreated, wild-type population of nematodes after a 48 h incubation, at which time adults and offspring were recovered.

Effect of DMSO on *C. elegans* reproduction

DMSO is commonly used as a solvent in many chemical exposure studies. Therefore it was critical to determine the maximum concentration of DMSO that will not affect *C. elegans* reproduction. Increasing concentrations of DMSO up to 5.5 % led to decreased numbers of offspring (Fig. 1), but were not lethal to the parents based on visual observations. The numbers of offspring decreased to 17 - 29 per parent at 2.5% DMSO, while only 1 - 3 offspring were recovered per parent at 5.5% DMSO (Fig. 1).

The concentration of a test chemical leading to a 50% reduction in *C. elegans* reproduction was calculated as the EC_{50}, with corresponding 95% confidence intervals. The concentration leading to no effect, defined as less than a 10% decrease in reproduction, was calculated as the benchmark concentration (BMC) with corresponding lower limits (BMCL).

Increasing the DMSO concentration caused the number of offspring to sharply decrease for both wild-type and *myo-2::GFP C. elegans*, with EC_{50}s ranging from 2 - 2.5% DMSO (Fig. 2, Table 1). BMCs ranged from 1.27 – 1.71% DMSO for wild-type and from 1.08 – 2.04% for *myo-2::GFP* nematodes. Based on these observations, DMSO concentrations ≤ 1% were selected for subsequent studies in which this solvent was used as a vehicle.

Effect of pH

Previous studies demonstrated that exposing *C. elegans* to low pH leads to decreased survival and movement (Khanna *et al.*, 1997; Cole *et al.*, 2004). Many chemicals are known to decrease the pH of exposure solutions, which could potentially confound the observed effects. Therefore, the pH range that does not affect *C. elegans* reproduction was determined. Figure 3 presents representative experiments showing fitted Hill functions for the effects of pH on reproductive counts for wild-type nematodes treated with ascorbic acid and *myo-2::GFP* nematodes treated with acetic acid. Calculated BMpH values ranged from 3.73 – 4.47 for wild-type nematodes treated with ascorbic acid and from 4.51 – 4.65 for *myo-2::GFP* nematodes treated with acetic acid (Table 2). For each acid, BMpHs and pH_{50}s were similar suggesting that there was a critical pH range below which reproduction would be severely inhibited. Based on these results, all reproduction assays were performed at pH > 4.5 to prevent confounding effects on chemical toxicity.

Effects of toxicants on *C. elegans* reproduction

Cadmium chloride was used as a model toxicant to evaluate whether results obtained from the Biosort were comparable to those made by manual counting of offspring. The number of offspring measured after 48-h cadmium exposures decreased in a concentration-dependent manner (Fig. 4) with EC_{50}s ranging from 176 – 192 μM for wild-type *C. elegans* (Table 3). These results are in good agreement with a previous report of an EC_{50} of 151 μM cadmium, measured by manual counting (Anderson *et al.*, 2001). The *myo-2::GFP* strain was slightly more sensitive than the wild-type strain to cadmium, with EC_{50}s ranging from 113 – 120 μM (Table 3). Although the *myo-2::GFP* strain was more sensitive to cadmium, the shapes of the concentration-response curves were similar with a sharp drop in reproduction observed between 100 and 200 μM cadmium (Fig. 4).

The effects on wild-type and *myo-2::GFP C. elegans* reproduction were examined with six additional chemicals including three pesticides diquat, paraquat, and parathion; the mutagen ethyl methanesulfonate (EMS); and two drugs caffeine and methadone (Table 3; Suppl. Fig. 1). The organophosphate pesticide, parathion, was at least two orders of magnitude more toxic than any chemical tested with EC_{50}s between 1.14 – 2.17 μM. Although the EC_{50}s for methadone were slightly higher than those for cadmium (207 – 380 μM vs. 176 – 192 μM), the BMCs for methadone were less than half of cadmium (56 - 63 μM vs. 132 - 169 μM) indicating that *C. elegans* is more sensitive to lower concentrations of methadone. A similar but more dramatic trend was observed after diquat exposure in which the EC_{50}s ranged from 683 – 797 μM, while the BMC was similar to methadone (54 – 62 μM). Paraquat was less toxic as judged by the EC_{50}s (1,677 – 2,346 μM) or BMCs (294 – 975 μM). Ethyl methanesulfonate exhibited low toxicity to *C. elegans* with EC_{50}s and BMCs between 4.66 – 4.77 and 3.77 – 4.12 mM, respectively. Caffeine was the least toxic of the chemicals tested as calculated by the EC_{50} of 8.74 – 12.65 mM; however, *C. elegans* reproduction was affected by lower concentrations of caffeine than EMS as measured by the BMC (1.84 – 3.43 mM).

Discussion

The *C. elegans* reproduction assay described in this report used the COPAS Biosort to count the number of offspring produced between the L4 larval stage and adulthood. This period was

chosen to coincide with the developmental stage when the number of germ cells reached its maximum, but before the embryonic membrane became impermeable (Anderson, 1995). Before testing chemical toxicity, preliminary experiments focused on optimizing the duration of the chemical exposure, the amount of bacterial food, and number of animals to be used. An exposure time of 48 h was chosen to maintain a relatively low number of offspring of 33-43 per untreated parent. This exposure time avoided overcrowding in the wells and allowed nematode counts to remain within the sampling limits of the Biosort. Chemical exposures were started at the L4 stage to maximize the chance of observing potential chemical effects on fertility, while avoiding potential effects on parental growth. When the offspring were sampled, adults had been producing embryos for approximately 36 h and still had several days of embryo production remaining.

Other studies have quantified the effects of a variety of chemicals on *C. elegans* reproduction (Dhawan *et al.*, 1999; Anderson *et al.*, 2001; Bull *et al.*, 2007; Sicard *et al.*, 2007). Traditionally, one to five parents are treated for 2 to 3 day incubation periods. At the end of the incubation, or every 24 h, the number of offspring is manually determined by visual observation. These methods can be tedious and imprecise. In addition, when nematodes are cultured in liquid medium, obtaining exact counts for moving larvae can be difficult. To determine the effects of hundreds to thousands of chemicals on reproduction an automated high-throughput system would be required. The chitinase assay, which indirectly measures reproduction by estimating the numbers of hatching embryos, may be applicable to HTS (Kaletta and Hengartner, 2006). This assay, however, gives only a snapshot of the number of embryos at a specific developmental stage, but does not include hatched larvae or less developed embryos. The reproduction assay described in this report directly measures the number of offspring in a rapid, automated fashion. Because the Biosort measures the size of each nematode, the population size distribution of offspring after parental exposure can also be quantified, which could provide further insight into the chemical's effects on growth and development (Boyd *et al.*, 2009; Smith *et al.*, 2009).

Because many organic chemicals have limited aqueous solubility, many chemical libraries such as the NTP's 1408 (Xia *et al.*, 2008) and the EPA's ToxCast 320 (Dix *et al.*, 2007) are prepared in 100% DMSO. For this reason, a concentration of DMSO that would not significantly affect *C. elegans* reproduction was determined. *C. elegans* reproduction was unaffected at DMSO concentrations below 1% DMSO, which confirmed that this solvent could be used as a vehicle in future *C. elegans* HTS.

Cadmium chloride was used to determine if Biosort data accurately reflect the effects of chemicals on *C. elegans* reproduction. Biosort EC_{50} values were compared to a previously published reproduction EC_{50} made by microscopic observations. An average EC_{50} of 185 μM for wild-type nematodes agreed with the previously reported value of 151 μM (Anderson *et al.*, 2001). In addition, these values are comparable to those observed in other *C. elegans* toxicological assays. For example, an EC_{50} of 122 μM cadmium for *C. elegans* feeding was previously reported (Boyd *et al.*, 2007).

An $EC_{50}$of 18 μM cadmium was previously reported for *C. elegans* reproduction using the Biosort (Boyd *et al.*, 2007). This difference may be attributed to the differences in the solvents used in the two studies. The previous study was performed using K-medium containing 1% DMSO (final concentration). The current study however was performed using completely aqueous media. The ability of DMSO to affect membrane permeability in toxicological assays has been well documented. Thus, DMSO may have affected cadmium's bioavailability by allowing it to easily pass through the nematode cuticle. An analogous phenomena has been observed in *C. elegans* mutants in which the cuticle is more permeable. These mutants are more

sensitive to chemical toxicity compared to wild type animals (Watanabe *et al.*, 2005; Partridge *et al.*, 2008).

Several potential mechanisms by which a chemical could reduce reproductive performance include: (1) diminished fertility, due to a decrease in oocyte or sperm production; (2) inhibited egg-laying, through neurotoxic effects on vulval neurons and muscles; or (3) reduced offspring survival, by impairing hatching or increasing embryonic lethality. The set of chemicals examined in this report have the potential to affect reproduction via several of these mechanisms.

The DNA alkylating agent EMS has been used as an effective chemical mutagen to generate point mutants in countless numbers of reverse genetic studies (Rosenbluth *et al.*, 1983). Parent nematodes are typically exposed to 50 mM EMS for several hours after which their offspring are screened for desired phenotypes. Although the exposure concentration used for creating mutants is much higher than the EC_{50} for reproduction (50 mM vs. 470 μM), the exposure duration is also much shorter (4 h vs. 48 h). EMS-induced DNA damage would be expected to lead to the production of non-viable sperm and oocytes, as well as decreased embryonic hatching and survival. This hypothesis is supported by the observation that hundreds of embryonic lethal and reproduction-deficient *C. elegans* mutants have been isolated during EMS screens.

The pro-oxidant herbicides paraquat and diquat cause toxicity via generation of intracellular superoxide anions. The production of reactive oxygen species can damage a variety of cell types including oocytes and sperm (McCarthy *et al.*, 2004; Salinas *et al.*, 2006). Because of this increase in oxidative stress, a range of paraquat exposure conditions have been used to investigate stress tolerance in *C. elegans* (Ishii *et al.*, 1990; Hartman *et al.*, 1995; Khare *et al.*, 2009; Nemoto-Sasaki and Kasai, 2009). Increased time to first egg-laying (Hartman *et al.*, 1995) and decreased fecundity (Khare *et al.*, 2009) have been observed in paraquat-exposed *C. elegans* at concentrations similar to those used in the current study (100-300 μM).

Parathion was the most potent inhibitor of reproduction, followed by cadmium and methadone. Methadone and parathion are potent disruptors of the central nervous system in humans acting by binding to opioid receptors (Garrido and Troconiz, 1999) and inhibiting cholinesterase activity (Aardema *et al.*, 2008), respectively. Cadmium-induced neurotoxicity is caused by a variety of mechanisms including oxidative neuropathy and disruption of neurotransmitter release (Méndez-Armenta and Ríos, 2007). Neurotoxic phenotypes are also observed in *C. elegans* exposed to cadmium. Cadmium inhibits *C. elegans* neuromuscular activity (i.e., movement) and causes ‘bagging’, a phenotype in which hermaphrodites fail to lay embryos and larvae hatch inside the parents (Freedman, unpublished observation). Egg-laying defects are generally caused by a failure of vulval function and have been observed following exposure to other neurotoxicants (Bany *et al.*, 2003; Tokuoka *et al.*, 2008). In addition to being a neurotoxicant, cadmium also increases germ cell apoptosis and decreases germ cell proliferation in *C. elegans* (Wang *et al.*, 2008). These various modes of cadmium toxicity would lead to a decrease in *C. elegans* reproduction. Although parathion and methadone exposures have not been previously described in *C. elegans*, they may also directly affect *C. elegans* egg-laying.

One of the goals for the development of alternative test species is their eventual application in the prediction of human toxicological responses. If *C. elegans* toxicological data is to be used to predict human toxicity, then the predictive power relative to traditional models of toxicity must be evaluated. To evaluate the *C. elegans* assay, the average reproduction EC_{50}s were listed in rank order from most to least toxic, as compared to previously reported mouse and rat LD_{50}s (Table 4). In the three species, parathion was found to have the highest toxicity followed

by cadmium and methadone. In rodents, EMS was the least toxic; however, in *C. elegans*, EMS was ranked sixth with caffeine being the least. The Spearman non-parametric correlation r-values were 0.8214 and 0.9286 when regressing *C. elegans* EC_{50}s against mouse and rat LD_{50}s, respectively, compared to an r-value of 0.8571 for mouse vs. rat LD_{50}s, with corresponding p-values of 0.0341, 0.0067, and 0.0238. Thus, an excellent correlation was observed between *C. elegans* and mice or rat toxicity. These correlations were at levels equal to that observed between mice and rats. Overall, *C. elegans* reproduction appears to be an excellent predictor of rodent lethality; however, more chemicals need to be tested to verify these results.

Conclusion

To assess the large number of chemicals that are lacking sufficient toxicity data, international agencies are exploring the use of high-throughput screening and testing using alternative model organisms. When using alternative model organisms it is necessary to consider the advantages and limitations of each system. The *C. elegans* reproduction assay is rapid, reproducible, and responses can be observed for a wide range of chemicals. In the future, *C. elegans* responses to additional toxicant exposures will need to be correlated with responses observed in other species and *in vitro* HTS assays.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Abbreviations

TOF	time of flight (nematode length)
EXT	extinction (optical density)
EC_{50}	half maximal effective concentration
BMC	benchmark concentration

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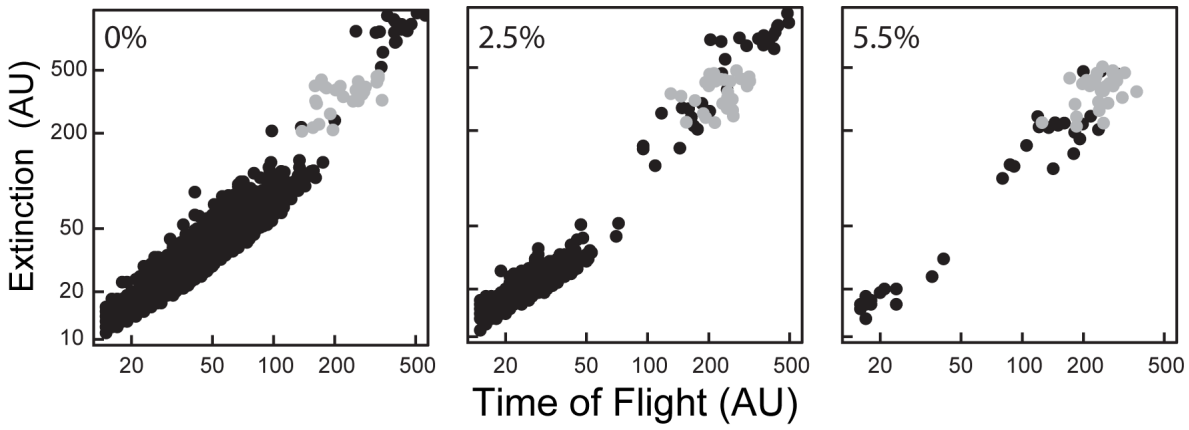

Figure 1. Scatter plots illustrating concentration-dependent decreases in *C. elegans* reproduction

Extinction ($\log_{10}$ scale) plotted against time of flight ($\log_{10}$ scale) for untreated (*left panel*), 2.5% DMSO (*center panel*), and 5.5% DMSO (*right panel*) wild-type nematodes. *C. elegans* L4s (*gray circles*) were loaded at t=0 h. After 48 h, the nematodes were recovered as adults and their offspring (*black circles*). Each point corresponds to a single nematode. (AU, arbitrary units)

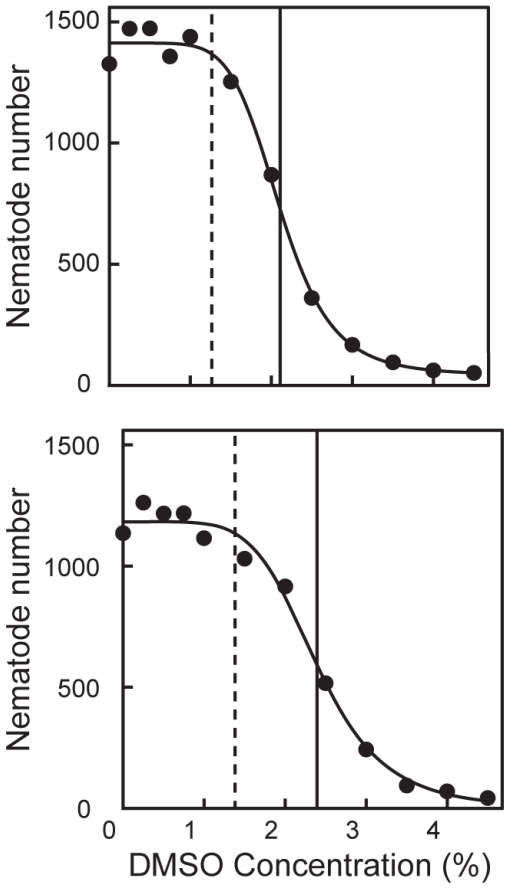

Figure 2. Effect of DMSO on *C. elegans* reproduction

Total number of offspring plotted against DMSO concentration (%) of wild-type (*upper panel*) and *myo-2::GFP* (*lower panel*) strains for one representative experiment. The solid, black vertical lines represent a 50% reduction in the total number of offspring (EC_{50}). The dashed, black vertical lines represent the BMC, below which the reproductive rate is not significantly affected by DMSO.

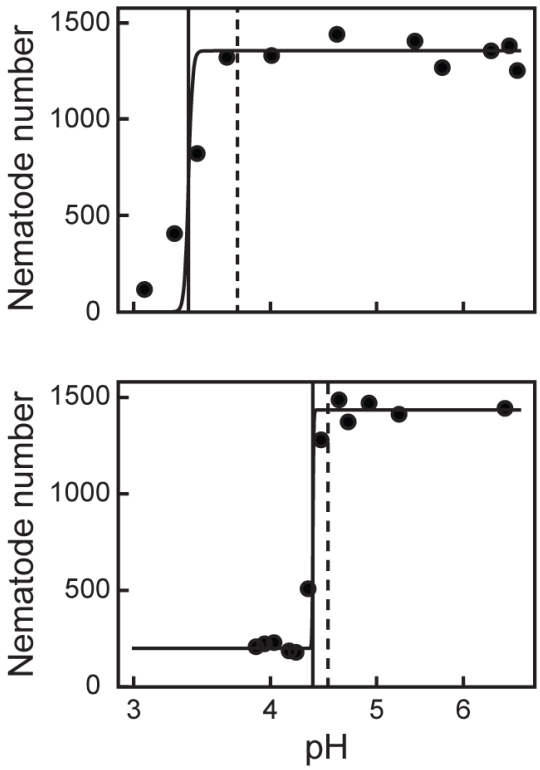

Figure 3. Effects of pH on *C. elegans* reproduction

Total number of offspring plotted against pH for one representative experiment. Increasing concentrations of ascorbic acid (*upper panel*) or acetic acid (*lower panel*) caused a decrease in the number of wild-type and *myo-2::GFP* offspring, respectively. The solid, black vertical lines represent a 50% reduction in the total number of offspring (pH_{50}). The dashed, black vertical lines represent the BMpH, above which the reproductive rate is not significantly affected by pH.

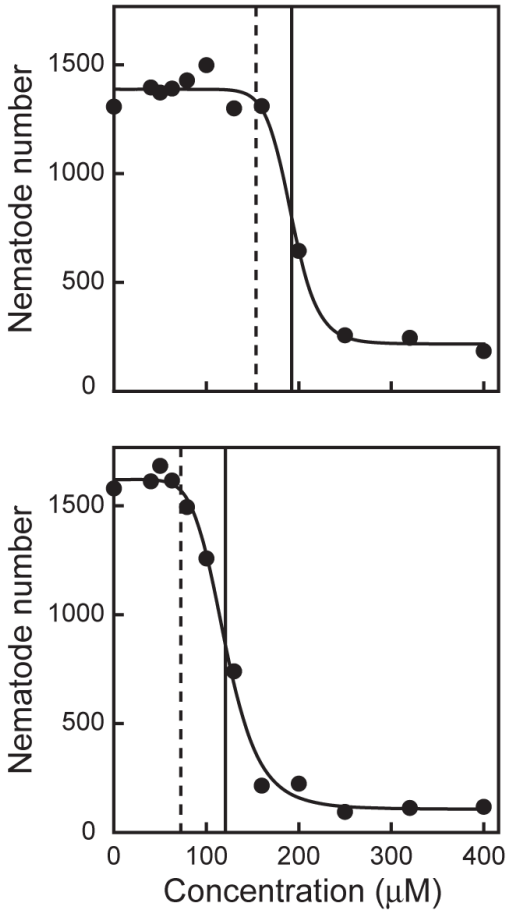

Figure 4. Effect of cadmium on *C. elegans* reproduction

Total number of offspring plotted against cadmium concentration (μM) of wild-type (*upper panel*) and *myo-2::GFP* (*lower panel*) strains for one representative experiment. The solid, black vertical lines represent a 50% reduction in the total number of offspring (EC_{50}). The dashed, black vertical lines represent the BMC, below which the reproductive rate is not significantly affected by cadmium.

Table 1

Effect of DMSO on *C. elegans* reproduction

Strain	EC_{50}	95% CI	BMC	95% BMCL (lower limit)
wild-type	2.11	(2.04, 2.18)	1.27	1.07
	2.21	(2.11, 2.32)	1.71	1.48
	2.39	(2.23, 2.56)	1.62	1.35
myo-2::GFP	2.01	(1.81, 2.20)	1.08	0.79
	2.52	(2.22, 2.83)	2.04	1.55
	2.39	(2.30, 2.49)	1.38	1.20

Values expressed as % DMSO

Table 2

Effect of pH on *C. elegans* reproduction

Acid	Strain	pH_{50}	95% CI	BMpH	95 % BMpH (upper limit)
Ascorbic acid	wild-type	3.70	(3.62, 3.78)	4.26	4.53
		3.37	(3.34, 3.40)	3.73	3.83
		3.81	(3.70, 3.93)	4.47	4.82
Acetic acid	*myo2::GFP*	4.41	(4.37, 4.45)	4.55	4.64
		4.45	(4.43, 4.47)	4.65	4.70
		4.37	(4.36, 4.38)	4.51	4.54

Table 3

Effects of toxicants on *C. elegans* reproduction

Chemical	Strain	EC_{50}	95% CI	BMC	95% BMCL (lower limit)
Cadmium	wild-type	186	(170, 201)	169	138
		176	(158, 194)	132	95
		192	(186, 198)	154	137
	myo-2::GFP	113	(109, 117)	81	72
		119	(112, 125)	89	77
		120	(116, 125)	72	63
Diquat	*myo-2::GFP*	756	(655, 858)	62	26
		797	(686, 907)	54	21
		683	(492, 875)	55	11
Paraquat	wild-type	1677	(1075, 2279)	294	64
		1739	(1473, 2005)	975	601
		2346	(2005, 2687)	463	232
Parathion	*myo-2::GFP*	2.17	(2.01, 2.32)	1.31	1.02
		1.22	(1.07, 1.36)	0.43	0.26
		1.14	(1.08, 1.20)	0.69	0.56
EMS	wild-type	4656	(4188, 5124)	3766	2908
		4666	(4347, 4986)	4115	3320
		4768	(4539, 4997)	4028	3465
Caffeine	*myo-2::GFP*	8903	(7584, 10223)	1845	1024
		12650	(11278, 14021)	3431	2063
		8739	(7160, 10318)	2091	1058
Methadone	wild-type	207	(192, 221)	63	40
		270	(255, 285)	57	36
		380	(340, 419)	56	27

Values expressed as μM

Table 4

Comparison of average *C. elegans* reproduction EC_{50}s with rodent oral LD_{50}s

Chemical	*C. elegans* EC_{50}[a]	Mouse LD_{50}[b]	Rat LD_{50}[b]
Parathion	1.51	5	2
Cadmium	185	60	88
Methadone	286	70	86
Diquat	745	233	120
Paraquat	1,921	120	150
EMS	4,697	470	350[c]
Caffeine	10,097	127	192

[a] EC_{50}s expressed as μM

[b] Oral LD_{50}s expressed in mg/kg (Lewis, 2004)

[c] Intraperitoneal injection LD_{50} expressed as mg/kg (Lewis, 2004)