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NTP Medium-throughput Toxicity Screen using *C. elegans* - WormTox

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NIEHS

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U.S. Department of Health and Human Services
National Institute of Health
National Institute of Environmental Health Services

Project Tasks

√ **Task 1.** Develop methods to measure the toxicity of developmental and neurological toxicants This task involves the development of computer and image analysis software for monitoring growth, size, reproduction and movement. It also requires development of a 96-well format for growth, dosing and toxicity testing.

√ **Task 2**. Expose *C. elegans* to at least 200 known or suspected developmental and/or neurological toxicants and determine changes in phenotypic characteristics (survival, size, growth, reproduction and

√ movement).

Task 3. Create and/or obtain GFP-based, stress-responsive transgenic *C. elegans* for improving sensitivity and specificity of toxicity screens. This task will also include the development of multi-dimensional (3-D, 4-D) computer imaging software to quantitatively measure the effects of

√ toxicant exposure on nervous system development.

Task 4. Use *C. elegans* microarray analysis and test a subset of chemicals from Task 2.

Task 5. Adapt methods for high throughput analysis to assess the toxicological responses in *C. elegans* in which each gene has been inactivated using RNA interference.

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Infrastructure

- Robotics and Worm Sorting
  - Titertek MAP C2 Agar Dispenser
  - Biomek FX
  - Biomek 2000
  - COPAS Biosort
    (Complex Object Parametric Analyzer and Sorter)

- Microscopes
  - Inverted, motorized
  - GFP dissecting
  - Zeiss Confocal

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Toxicological Tests

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Standard Assay Protocol

- 12 concentrations, geometric dose-response
- n = 7 for each concentration
- Each assay is repeated a minimum of three times
- Growth
- Feeding
- Reproduction/Fecundity
- One month per chemical
- Six chemicals per month

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Chemicals Tested

Ref #		CAS #	NTP studies
1	Acetaminophen*	103-90-2	T,C,R,G
2	Acetic Acid	64-19-7	G
3	a-Cyclodextrin	10016-20-3	D
4	AgNo3	7761-88-8	N/A
5	AlCl3	10124-27-3	N/A
6	All-trans Retinoic Acid	302-79-4	D
7	Ascorbic acid**	50-81-7	T,C,R,G
8	AsNaO2	7784-46-5	I
9	b-Cyclodextrin	7585-39-9	N/A
10	bCyclodextrin hydrate	68168-23-0	N/A
11	Caffeine	58-08-2	T,R,D,G
12	Carbaryl	63-25-2	D
13	CdCl2	10108-64-2	I,G
14	Chlorpyrifos	2921-88-2	R,I,G
15	CoCl2	7646-79-9	N/A
16	CrO3	1333-82-0	N/A
17	CuSO4	7758-99-8	T,G
18	Demeton-S-methylsulfone	17040-19-6	N/A
19	Dichlorvos	62-73-7	T,C,G
20	Diphenylhydantoin	57-41-0	T,C,G
21	Diquat	6385-62-2	T
22	DMSO	67-68-5	G
23	EMS	62-50-0	D,G
24	ENU	759-73-9	D,G
25	Ethephon	16672-87-0	N/A
26	EtOH	64-17-5	C,R,D,G
27	Fipronil	120068-37-3	N/A
28	Fumonisin	116355-83-0	T,C,D,G
29	Glyphosate*	1071-83-6	T,G

Ref #		CAS #	NTP studies
30	Juglone	481-39-0	G
31	Lindane	58-89-9	T,C,G
32	Mercuric Chloride	7487-94-7	T,C,G
33	Metam Sodium	6734-80-1	N/A
34	Methadone hydrochloride	1095-90-5	I
35	Methanol	67-56-1	G
36	Methyl cellulose	9004-67-5	N/A
37	Methyl Mercury	16056-34-1	N
38	Methyl Parathion	298-00-0	T,C,G
39	Methylisothiocyanate	556-61-6	N/A
40	MMS	66-27-3	D,G
41	MNNG	70-25-7	D,G
42	Monocrotophos	6923-22-4	N/A
43	MoO3*	1313-27-5	T,C,G
44	Na2SeO3	10102-18-8	T
45	Nicotine	54-11-5	G
46	NiSo4 heptahydrate	10101-98-1	T,C,G
47	Paraquat	1910-42-5	G
48	Parathion	56-38-2	T,C,G
49	Pb(CH3COO)2•3H2O*	6080-56-4	R
50	PbNO3*	10099-74-8	N/A
51	PCB mixture*	TEFPCBMIX	C
52	PEG-60	25322-68-3	G
53	Pyridine	110-86-1	T,C,G
54	Tamoxifen*	54965-24-1	R
55	Tebuconazol	107534-96-3	D
56	Thimerosal	54-64-8	G
57	V2O5*	1314-62-1	T,C,I,G
58	Valproic acid	99-66-1	G

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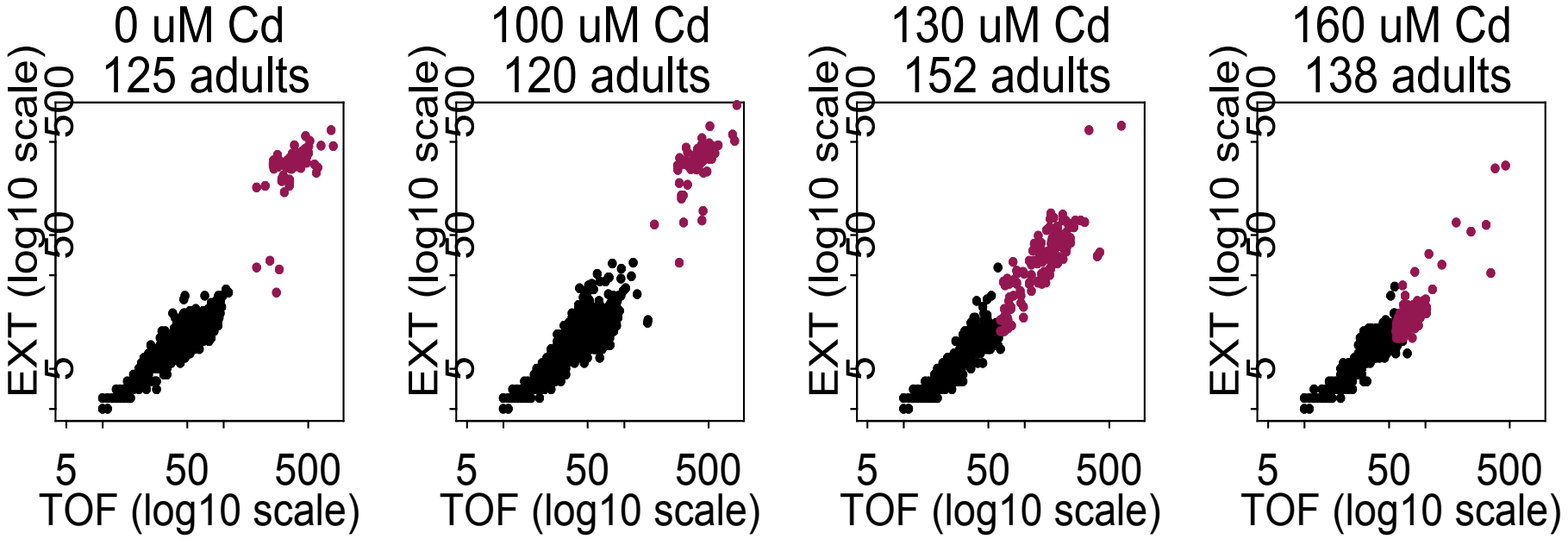
Effect of Cadmium on Growth



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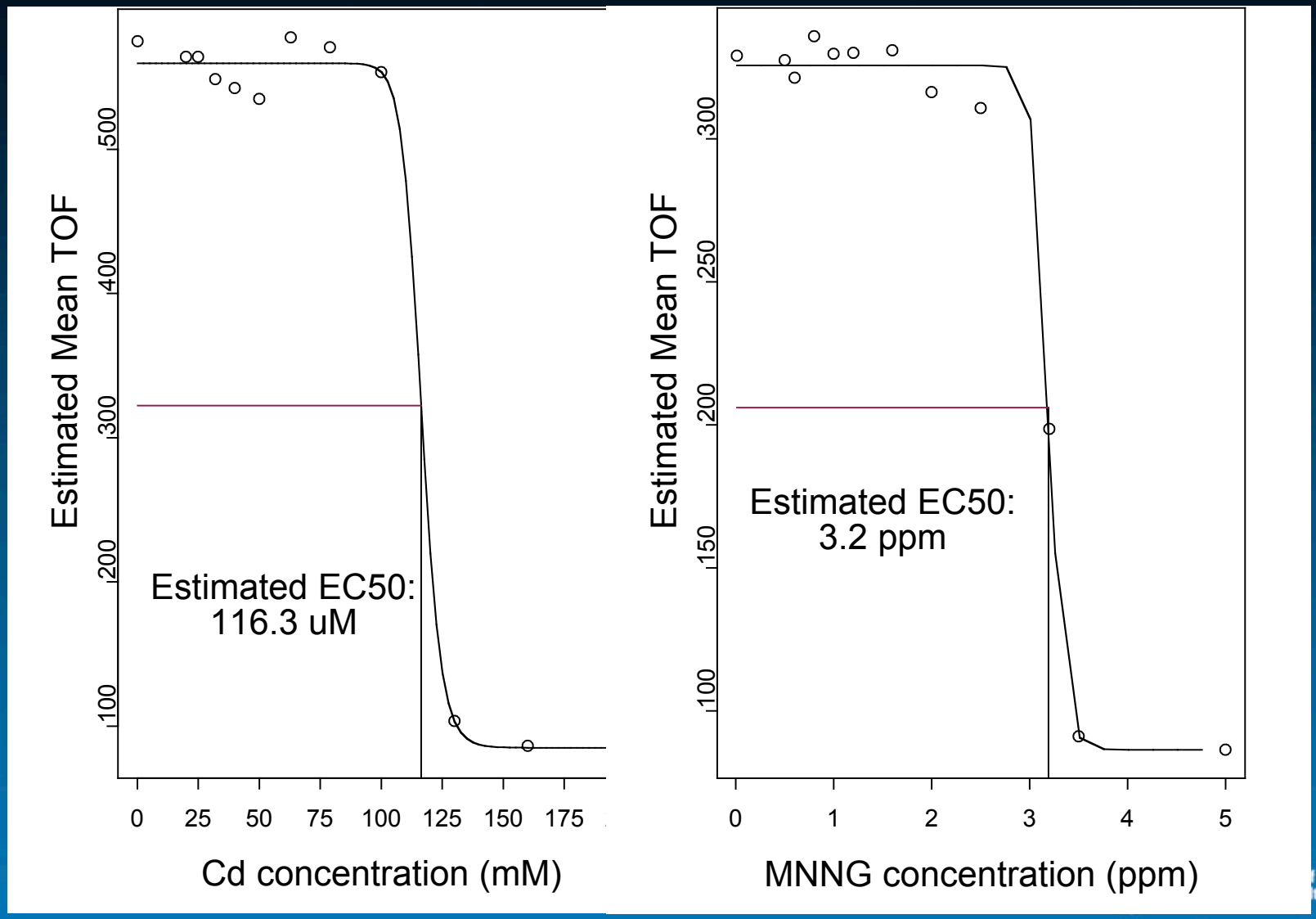
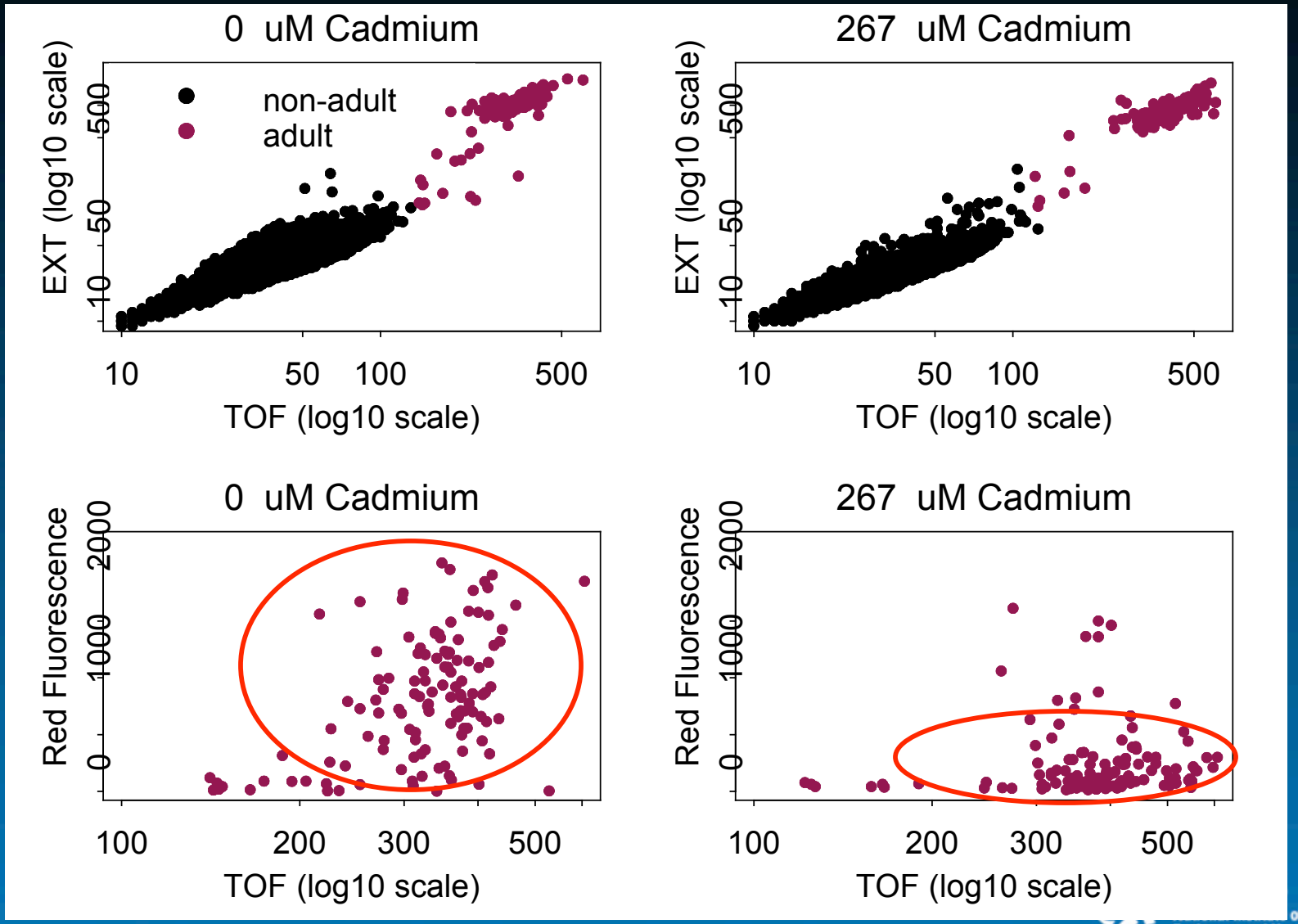
Growth: Dose - Response



Effect of Cadmium on Feeding



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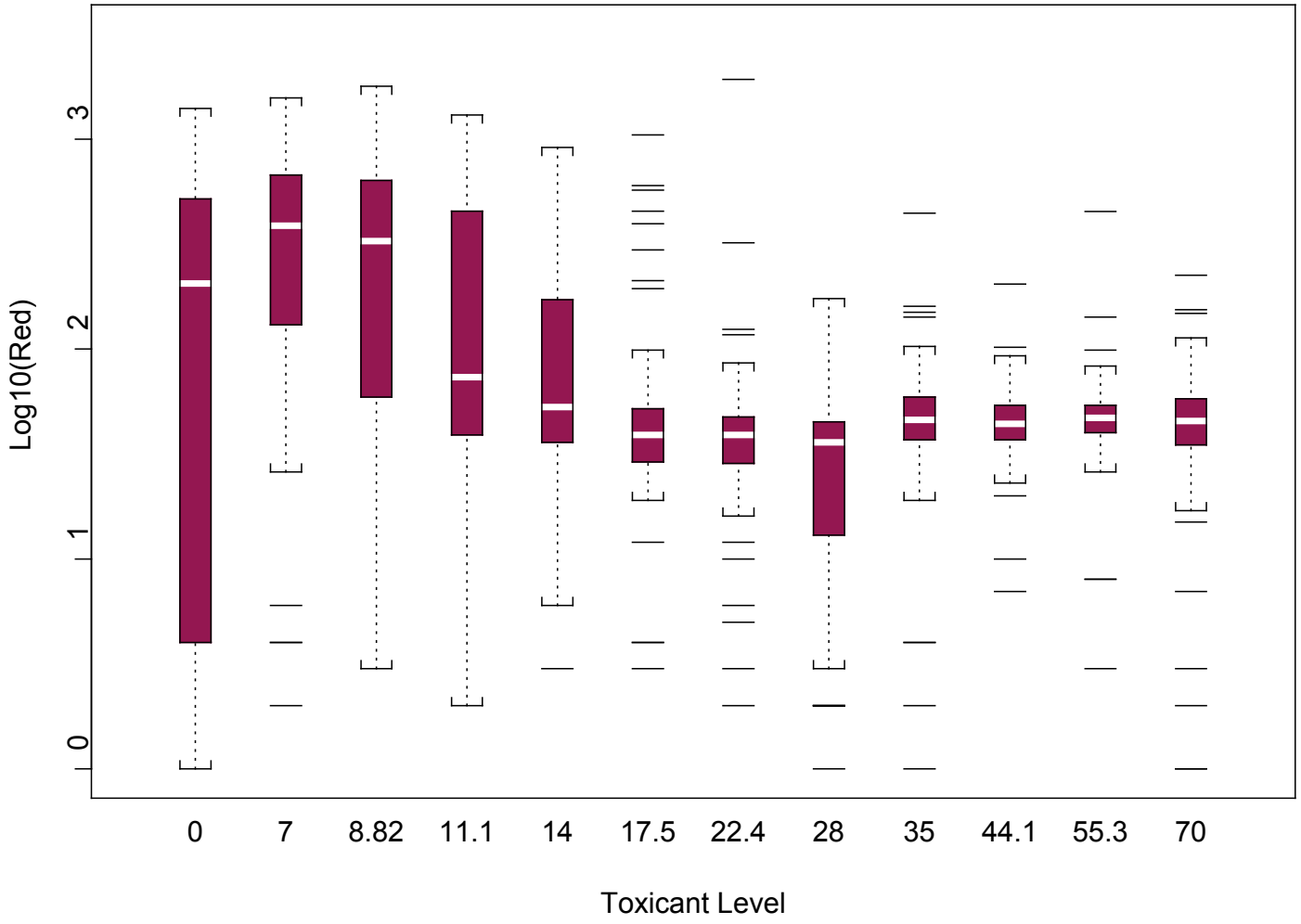
Effect of MMNG on Feeding



Transgenic Nematodes (GFP-based)

- Metal responsive: *cdr-1, mtl-1, mtl-2*
- Phase I and II Biotransformation
  - CYPs: 80 CeCYPs (e.g. cyp-35(A1-A5,B1-2,C1), cyp-31A, cyp-34A, cyp-29A)
  - Cyt b5: vem-1
  - GSTs: 36 CeGSTs
  - UDPGTs: 23 CeUDPGTs
  - Carboxylesterases: 17 Ce
- Apoptosis: *egl-1, ced-3, csp-1, csp-2, csp-3*
- Heat Shock Protiens: 22 CeHSPs
- Vitellogenins: *vit-1* to *vit-6*
- Acetylcholinesterases: *ace-1, ace-2, ace-3, ace-4*
- MAP Kinases: *mek-1, pmk-1*

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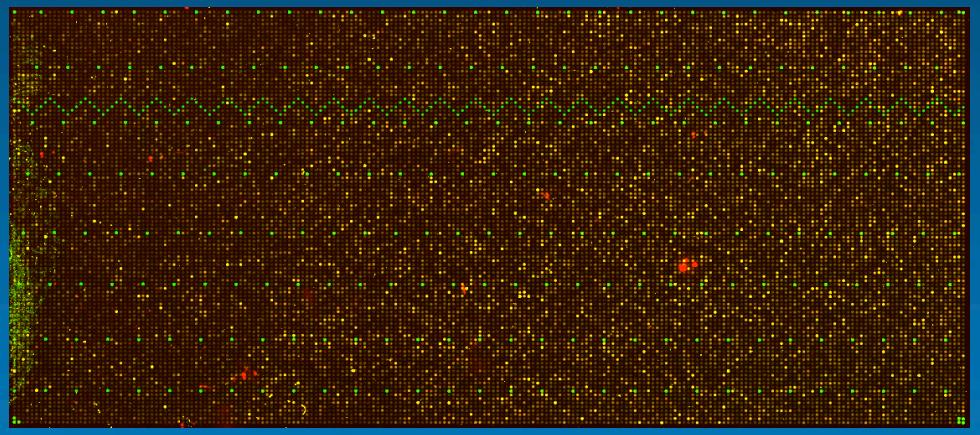
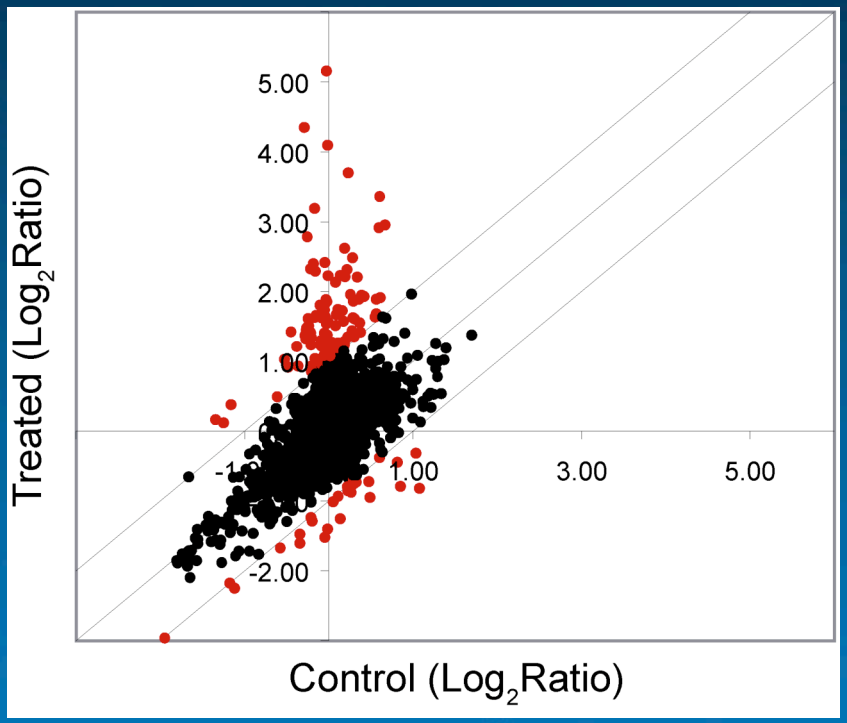
Genomics

- Agilent *C. elegans* custom microarrays
  - Based on ~21K predicted OFRs

    - Cadmium
    - NMMG
    - Diquat
    - Fumonisin





Current Developments

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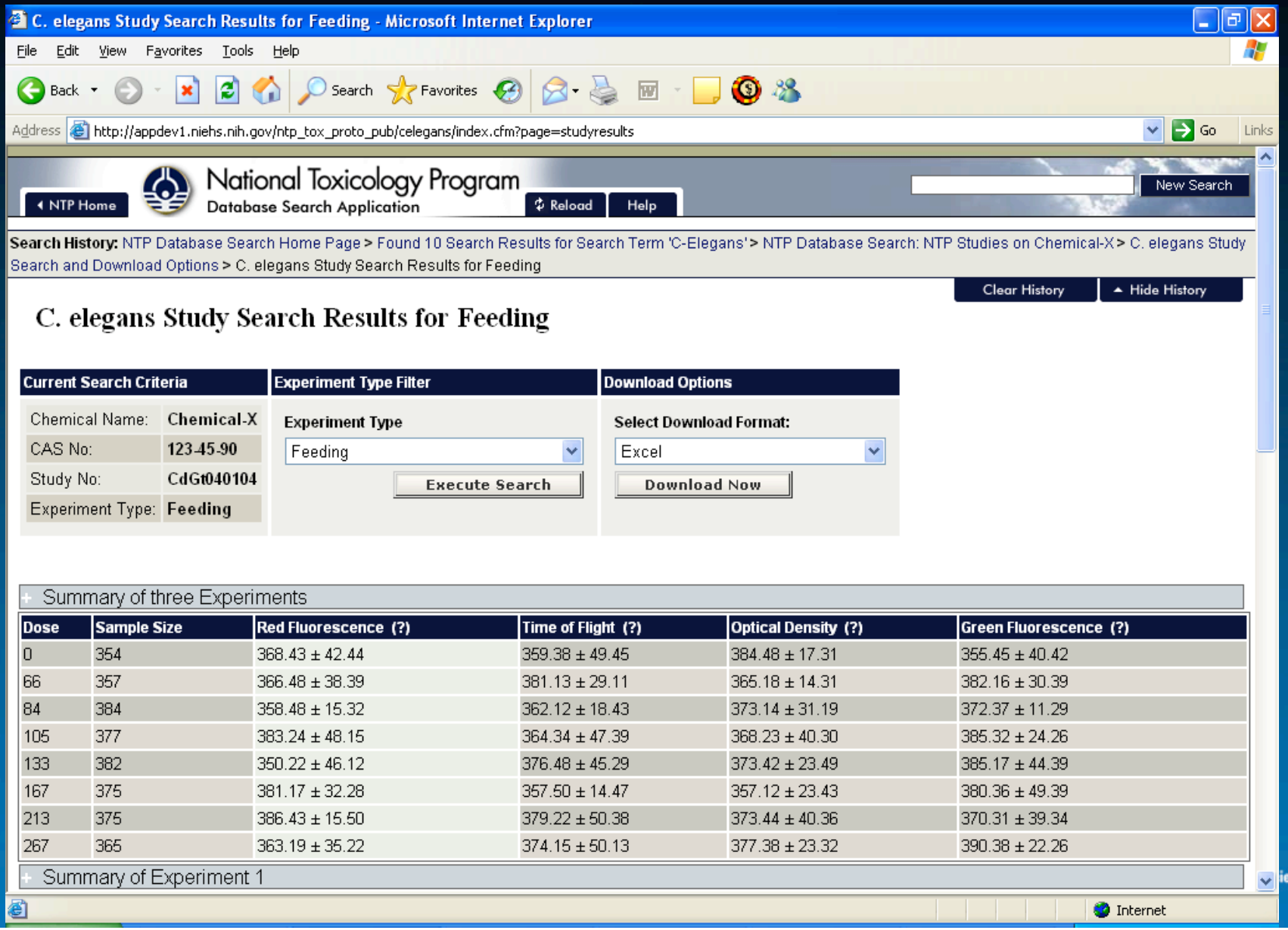
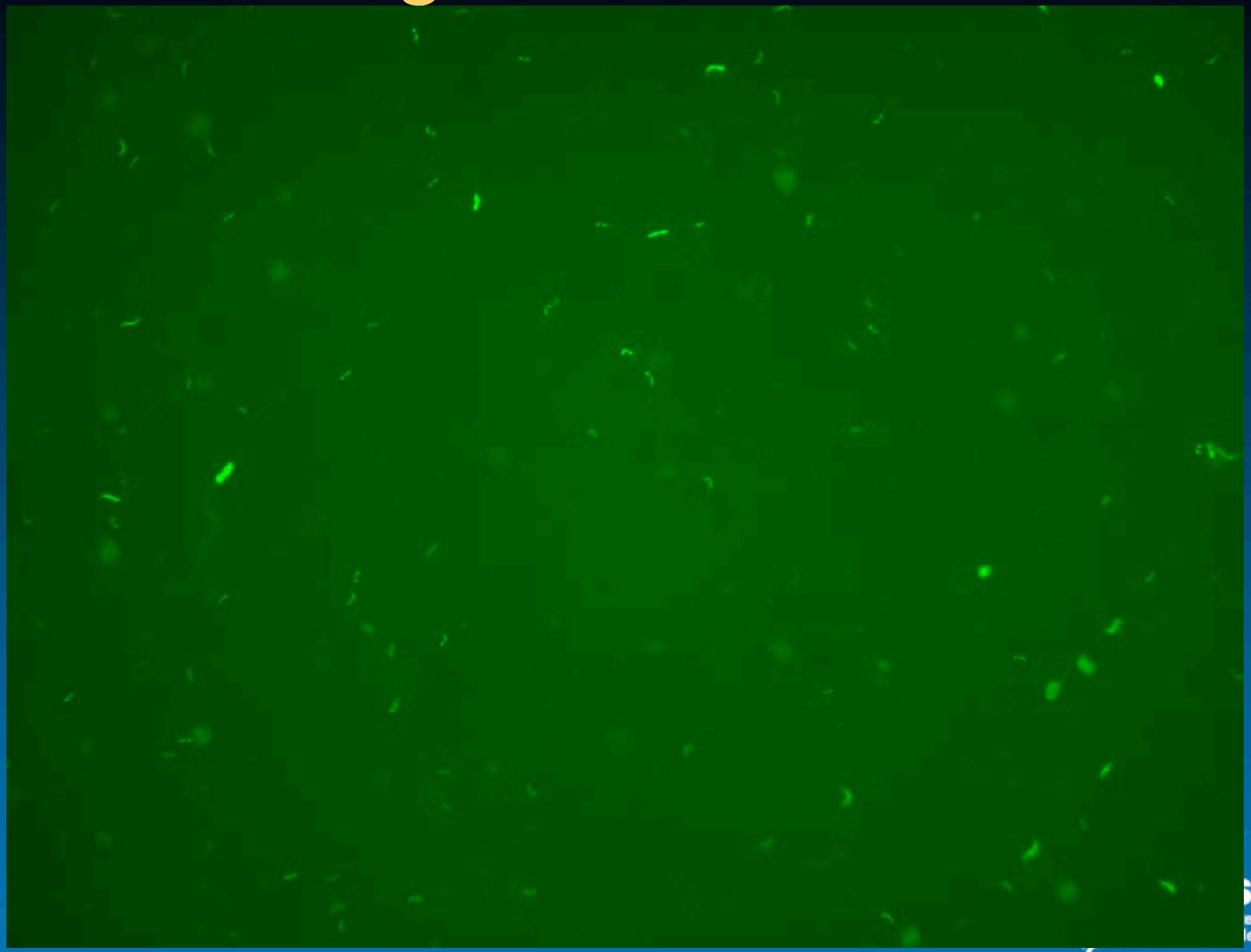
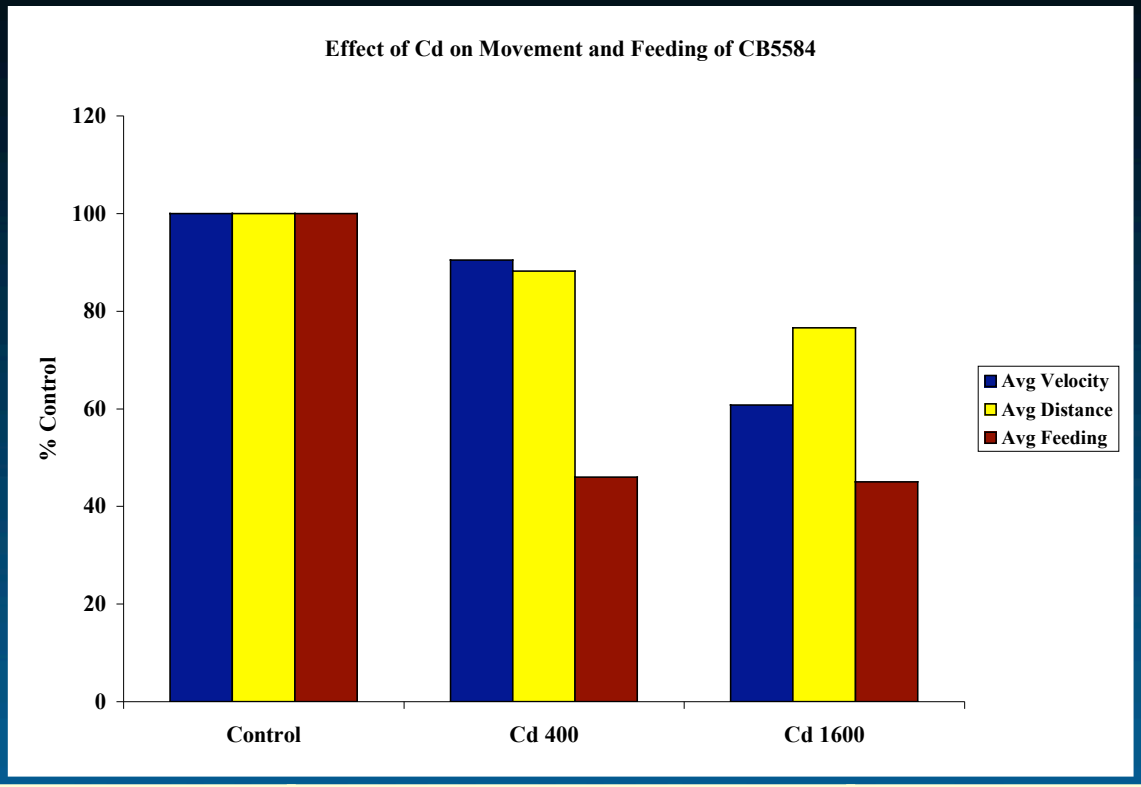
Public Accessible Database

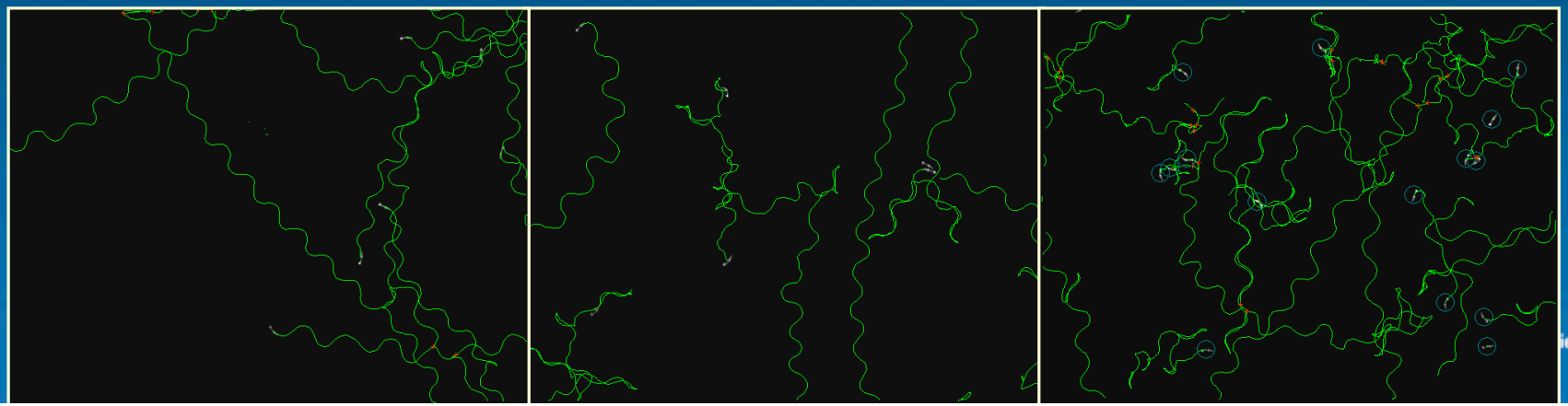


C. elegans in Motion



Effect of Cadmium on Movement





Alternative Tracking Software

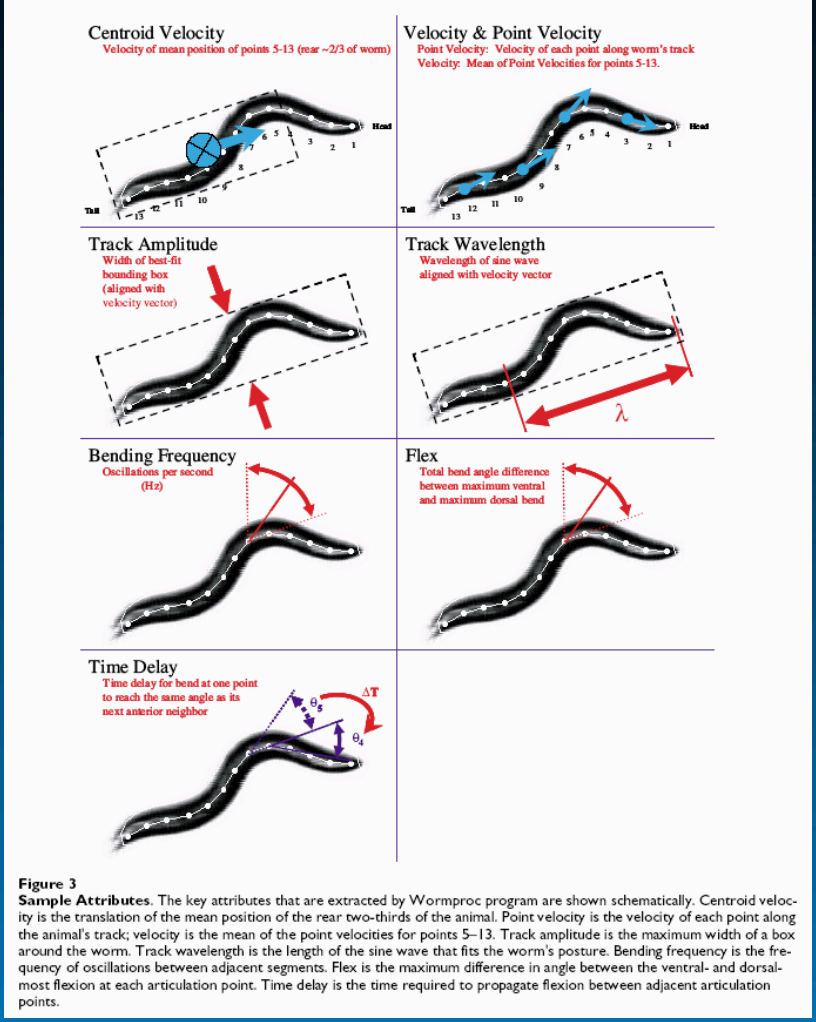
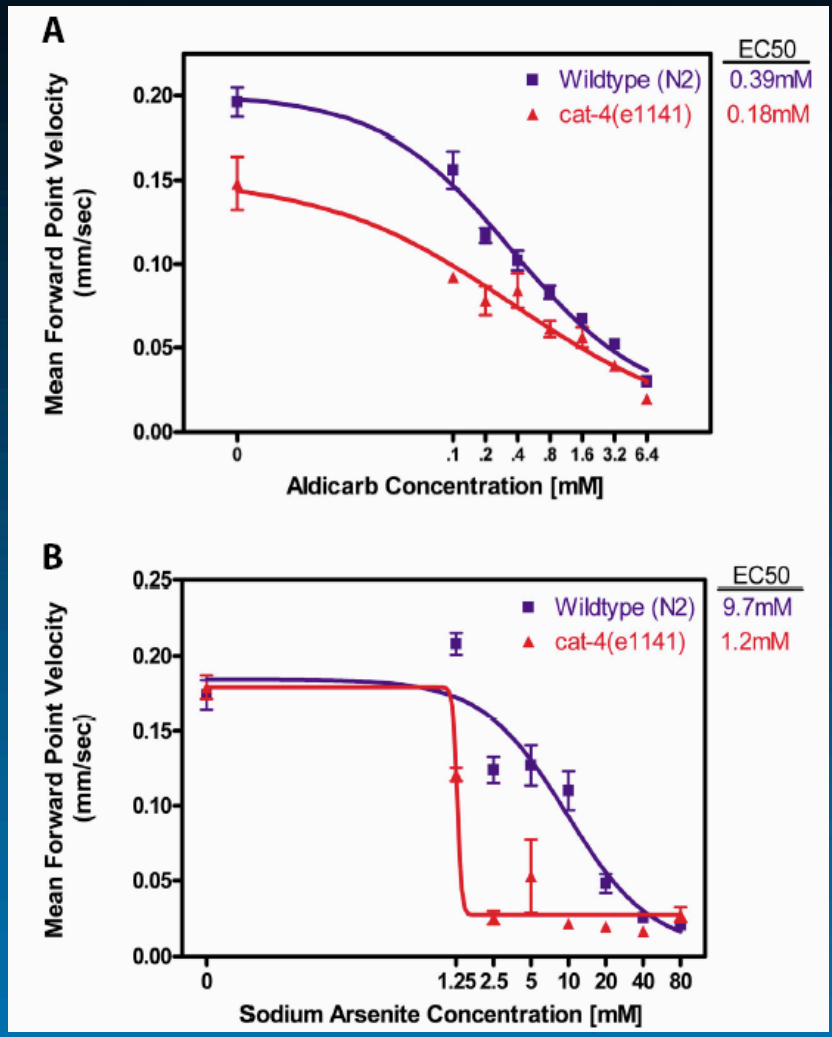


Figure 3
Sample Attributes. The key attributes that are extracted by Wormproc program are shown schematically. Centroid velocity is the translation of the mean position of the rear two-thirds of the animal. Point velocity is the velocity of each point along the animal's track; velocity is the mean of the point velocities for points 5–13. Track amplitude is the maximum width of a box around the worm. Track wavelength is the length of the sine wave that fits the worm's posture. Bending frequency is the frequency of oscillations between adjacent segments. Flex is the maximum difference in angle between the ventral- and dorsal-most flexion at each articulation point. Time delay is the time required to propagate flexion between adjacent articulation points.



Cronin et al. 2005

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Summary of Results with HTS Chemicals

- Completed two runs of the 1408
  - Three months for completion
  - 61 hits for both replicates
  - 182 hits for one replicate

- Future Development
  - Start HTS at 10 μM?
  - One more replicate at 100 μM?
  - Generate dose-response curves for hits

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100 μM HTS - Top 25 Hits

Chemical_Name	Chem Num	Counts	Perc	Pval	Counts	Perc	Pval
p-Toluenesulfonamide	1089	5	-0.974	0.131	14	-0.932	0.167
Actein	1102	7	-0.963	0.124	4	-0.981	0.137
Chlorpheniramine maleate	457	10	-0.952	0.141	5	-0.971	0.358
Isobutyl alcohol	997	15	-0.946	0.125	9	-0.96	0.129
Ninhydrin	951	13	-0.944	0.133	27	-0.857	0.146
N-Acetyl-p-toluidine	323	10	-0.942	0.13	22	-0.893	0.374
Cyclohexene oxide	1182	11	-0.941	0.118	13	-0.936	0.131
Hexyl cinnamic aldehyde	167	16	-0.933	0.319	20	-0.891	0.327
1,3-Dimethyl-4-nitrobenzene (4-Nitro-m-xylene)	579	18	-0.928	0.135	18	-0.903	0.137
Colchicine	1221	14	-0.922	0.125	9	-0.942	0.153
5-Methyl-2-nitroaniline	644	18	-0.922	0.141	21	-0.912	0.148
Adiponitrile	372	18	-0.915	0.125	94	-0.513	0.32
Cobaltocene	942	20	-0.914	0.138	19	-0.899	0.144
Lauryl chloride	1014	20	-0.911	0.145	62	-0.777	0.152
Glutaraldehyde (Glutaric dialdehyde)	945	17	-0.91	0.128	23	-0.901	0.137
Sodium lauryl sulfate	1200	20	-0.901	0.135	25	-0.883	0.136
a-Solanine	1207	21	-0.901	0.161	13	-0.935	0.161
boron trifluoride dihydrate	171	26	-0.891	0.33	16	-0.913	0.331
2-Amino-4-phenylthiazole HBr H2O	746	24	-0.889	0.158	86	-0.661	0.224
n-Octylamine	185	21	-0.885	0.324	15	-0.937	0.338
Triton X-100	1224	23	-0.872	0.136	28	-0.821	0.139
2-Nitropropane	456	27	-0.871	0.14	40	-0.771	0.314
7-Methylquinoline	720	35	-0.865	0.138	87	-0.674	0.212
1,3-Dinitronaphthalene	537	29	-0.855	0.13	35	-0.812	0.195
Pentachlorophenol	378	34	-0.84	0.14	131	-0.321	0.352

Analytical Challenges in Analyzing Data from WormTox Studies

- Goal: Model nematode populations using a 4-dimensional distribution
  - Time of Flight (TOF): length
  - Extinction (EXT): optical density
  - Fluorescence: green, red or yellow

- Develop statistical algorithms to:
  - Classify nematodes into discrete growth stages
  - Characterize statistical properties of nematode populations at different developmental stages and toxicant exposures
  - Rapidly analyze large volumes of data

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Manuscripts

- Meyer, J.N., Boyd, W.A., Azzam, G.A., Haugen, A.C., Freedman, J.H., and Van Houten, B. Decline of nucleotide excision repair capacity in aging *Caenorhabditis elegans.* (Submitted Genome Biol.)
- Alper, S., McBride, S.J., Lackford, B ., Freedman, J.H., Schwartz, D.A. Specificity and Complexity of the *C. elegans* innate immune response. (Submitted. Proc. Nat. Acad. Sci. U.S.A.)
- Cui,Y., Boyd, W.A., McBride, S.J., and Freedman, J.H. Functional Analysis of Cadmium Responsive Transcription in *Caenorhabditis elegans*. (To be submitted Genes & Develop.)
- Boyd, W.A., McBride, S.J., Rice, J.R., Snyder, D.W., and Freedman, J.H. Development of a feeding assay for medium-throughput toxicant screening using *C. elegans*. (To be submitted to Nat. Methods.)

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Future Directions

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Chemicals

- EPA Priority Chemicals (David Dix, EPA)
- EPA DNT Chemicals (Kevin Crofton, EPA)
- Marine toxins
- Ionic liquids (Michelle Hooth, NTP)
- Nano-materials (Nigel Walker, NTP)
- NTP 1408 (ver. 2)

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New Transgenic Strains

1. Wnt pathway via β–catenin and JNK
2. Receptor serine/threonine kinase (TGF-β receptor) pathway
3. Receptor tyrosine kinase pathway (small G-protein [Ras] linked)
4. Nuclear hormone receptor pathway
5. Notch–Delta pathway
6. Stress responses and checkpoints for DNA damage and replication.
7. Receptor-linked cytoplasmic tyrosine kinase (cytokine) pathway
8. Integrin pathway
9. Cadherin pathway
10. Gap junction pathway
11. Ligand-gated cation channel pathway
12. A stress response: The unfolded protein response (UPR)
13. G-protein coupled receptor (large G-protein) pathway
14. Apoptosis pathway (cell death pathway)
15. Receptor protein tyrosine phosphatase (RPTPs) pathway
16. Receptor guanylate cyclase pathway

SCIENTIFIC FRONTIERS IN DEVELOPMENTAL TOXICOLOGY AND RISK ASSESSMENT

Committee on Developmental Toxicology

Board on Environmental Studies and Toxicology

Commission on Life Sciences

National Research Council

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High Throughput Screening

- Goal: identify “hits” using vehicle control and positive control information
- Issues
  - Variability of reproduction counts
    - Day to day, plate to plate, within plate
  - Balance between # vehicle control wells & chemical reps
  - Choice of number of nematodes,
  - Exposure time
  - Positive controls: single concentration or dose-response curve
  - Carryover rates and number of rinse wells

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Long Term Directions

- Continued support for DIR and NTP studies
- Transcriptome analysis
  - Currently all transcriptomics are DIR-based research projects
  - Should WormTox collect microarray data for other chemicals?
- The function of WormTox in the NTP
  - Is it “Research and Development” or “Manufacturing”
  - Put out contract request for others to do WormTox activities

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The WormTox Group

- Windy Boyd
- Paul Dunlap
- Julie Rice
- Dan Snyder

- Sandra McBride

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