DTU Environment
Department of Environmental Engineering

AARHUS UNIVERSITY
DEPARTMENT OF ENVIRONMENTAL SCIENCE

MODUM
TOWARDS THE MONITORING OF DUMPED MUNITIONS THREAT

Environmental toxicity of chemical warfare agents in the Baltic Sea

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NATO OTAN
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Introduction

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› Chemical warfare agents (CWAs)
- Chemicals that due to their toxic properties injure, incapacitate or kill the target
- Prohibited by the Geneva Convention in 1925
- CWAs killed or injured 1.2 million people in World War I
- End of World War II: 65.000 tonnes CWAs produced by Germany alone

› Environmental concerns
- Large amounts dumped at sea (>11.000 t)
- Most munition cases expected to be corroded

What are their environmental toxicity?

Introduction cont'd

Map: Dumpsites in the Baltic Sea. Source: CHEMSEA, 2014

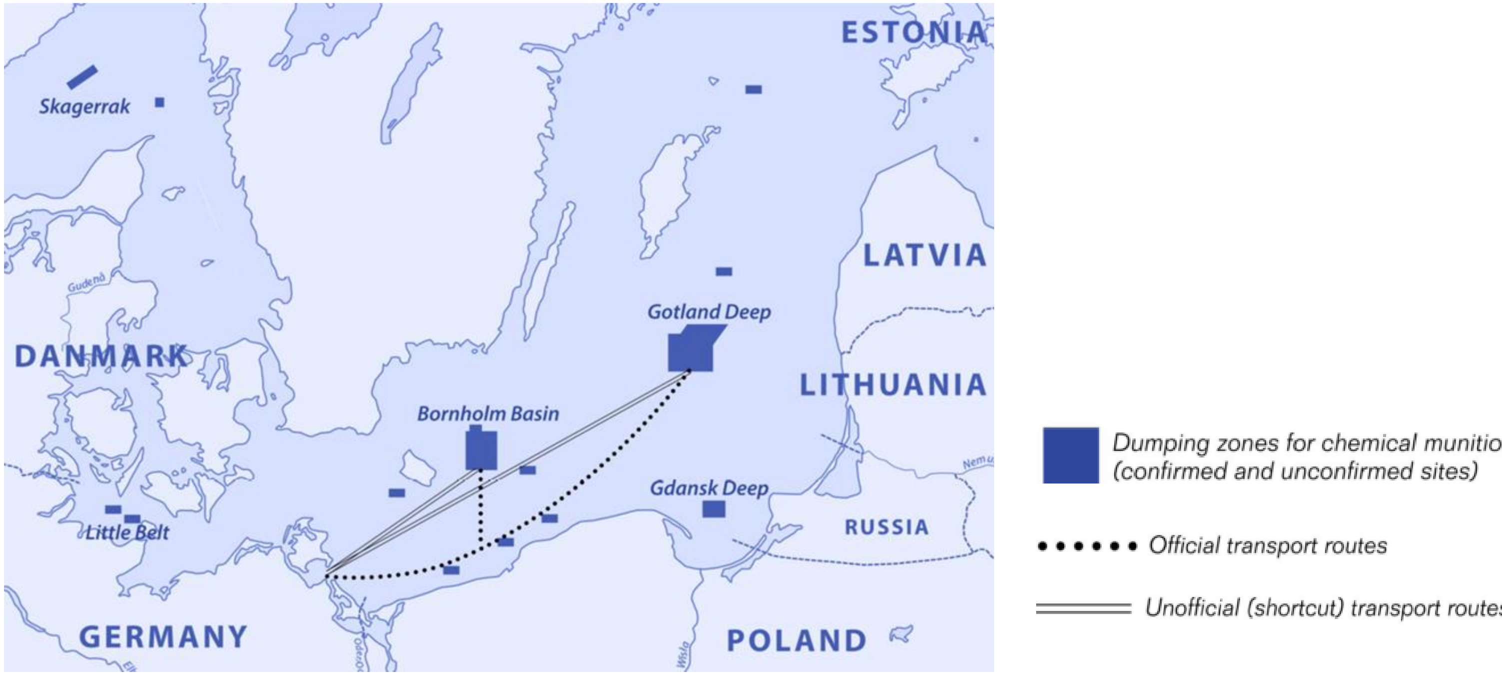


Table: Confirmed dumped CWAs in the Bornholm dumpsite east of Bornholm. It is the largest dumpsite in the Baltic Sea in terms of the amount of dumped munitions. Source: HELCOM, 1994.

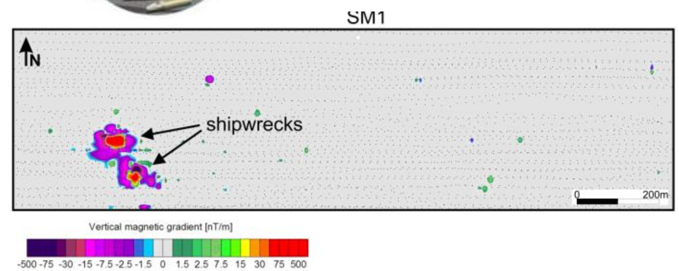
Compound	CAS #	Dumped [tonnes]
Sulfur mustard gas (Yperite)[1]	505-60-2	7027
Adamsite[2]	578-94-9	1428
Monochlorobenzene[3]	108-90-7	1405
Phenyldichloroarsine (PDCA)[2,4]	696-28-6	1017
Clark I (DPA)[2,4]	712-48-1	711.5
α-Chloroacetophenone (CAP)[5]	532-27-4	515
Triphenylarsine (TPA)[2,4]	603-32-7	101.5
Trichloroarsine (TCA)[2,4]	7784-34-1	101.5
Zyklon B[6]	74-90-8	74

[1]Blistering agent, [2]Organoarsenic blistering agent, [3]Additive, [4]Arsine oil constituent, [5]Riot control agent, [6]Blood agent.

MERCW (2005-2008)

Modelling of Environmental Risk related to sea-dumped Chemicals

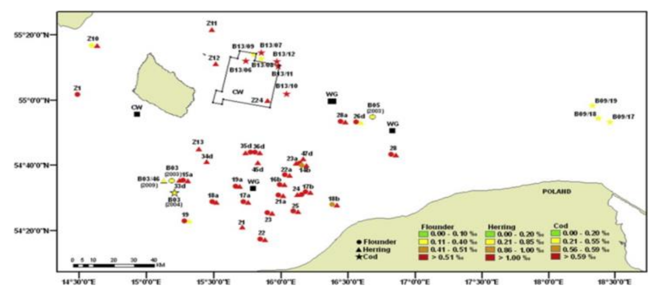
- Geophysical and magnetic analysis to locate munitions + sampling
- 8 metabolites of CWAs found – 2 of them in pore water



CHEMSEA (2011-2014)

Chemical Munitions Search and Assessment

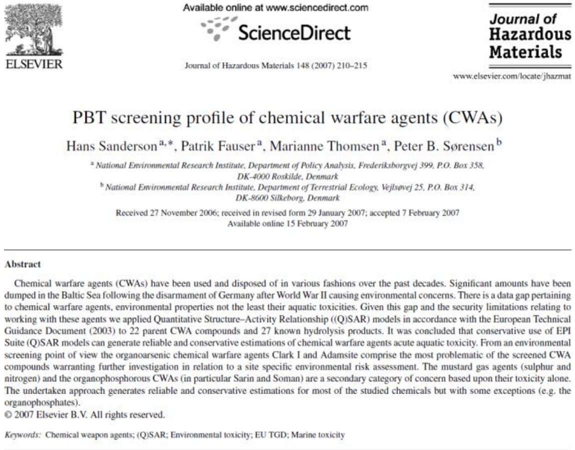
- Sampling of fish, mussels, sediment and pore water
- Improved analytical methods:
- 6 CWA metabolites detected in pore water



Nord Stream pipeline construction (2008-2012)

- 391 sediment and 11 pore water samples taken outside Bornholm dumpsite
- Predominantly organoarsenicals founds

Effects assessment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PBT screening profile of chemical warfare agents (CWAs)

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Abstract

Chemical warfare agents (CWAs) have been used and disposed of in various fashions over the past decades. Significant amounts have been dumped in the Baltic Sea following the disarmament of Germany after World War II causing environmental concerns. There is a data gap pertaining to chemical warfare agents, environmental properties not the least their aquatic toxicities. Given this gap and the security limitations relating to working with these agents we applied Quantitative Structure–Activity Relationship ((Q)SAR) models in accordance with the European Technical Guidance Document (2003) to 22 parent CWA compounds and 27 known hydrolysis products. It was concluded that conservative use of EPI Suite (Q)SAR models can generate reliable and conservative estimations of chemical warfare agents acute aquatic toxicity. From an environmental screening point of view the organoarsenic chemical warfare agents Clark I and Adamsite comprise the most problematic of the screened CWA compounds warranting further investigation in relation to a site specific environmental risk assessment. The mustard gas agents (sulphur and nitrogen) and the organophosphorous CWAs (in particular Sarin and Soman) are a secondary category of concern based upon their toxicity alone. The undertaken approach generates reliable and conservative estimations for most of the studied chemicals but with some exceptions (e.g. the organophosphates).

Keywords: Chemical weapon agents; (Q)SAR; Environmental toxicity; EU TGD; Marine toxicity

- Lack of CWA ecotoxicological data
- Use QSAR model –since mostly found organoarsenicals we can use inorganic arsenic as a conservative surrogate for extrapolation

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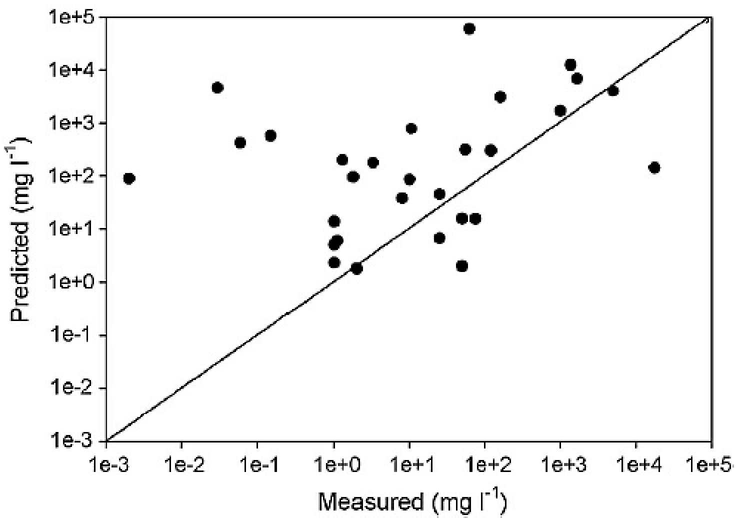

Fig. 1. Measured vs. predicted LC50 values—species specific (mg l^{-1}).

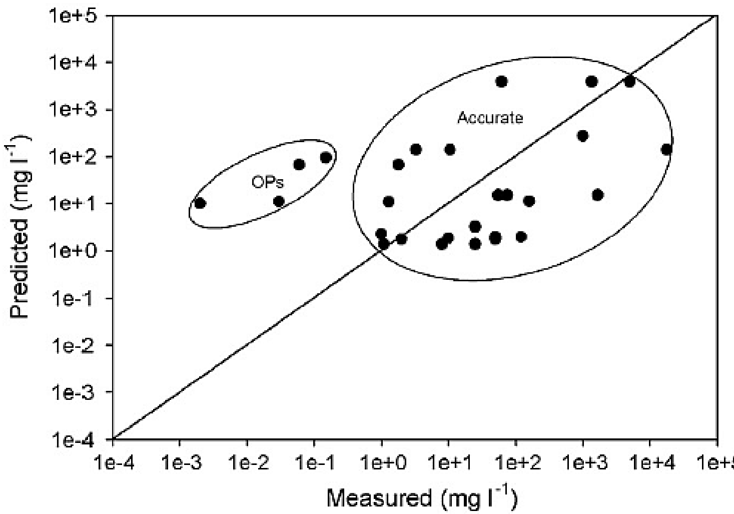

Fig. 2. Measured vs. predicted LC50—not species specific (lowest predicted) (mg l^{-1}).

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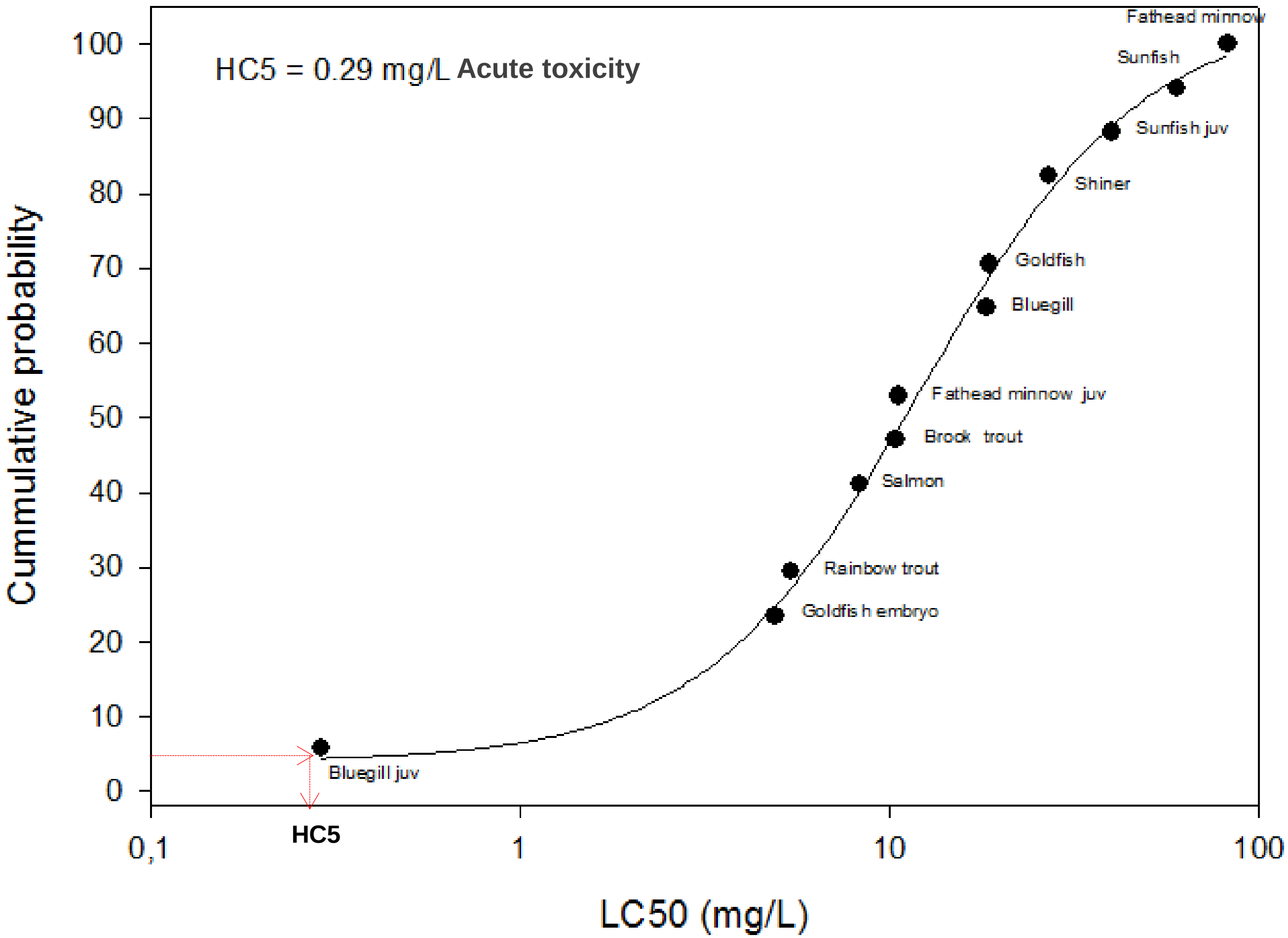
Organoarsenic fish community SSD



Measured/predicted environmental concentrations of detected compounds

Compound	CAS#	Sediment fraction		Pore water fraction		Source project
		Max. conc. [µg/kg]	DF [%]	Max. conc. [µg/L]	DF [%]	
Parent compounds:						
α-Chloroacetophenone (CAP)	532-27-4	7.5	1.1	0.92*		CHEMSEA
Triphenylarsine (TPA)	603-32-7	1,200 81,250	8.7 32.8	68 0.069*	2.7	CHEMSEA MERCW
Diphenylarsinic acid (DPA[ox])	4656-80-8	1,700 9,583 140	8.2 50 19.5	940 1,538 6.6*	2.2 5.2	CHEMSEA MERCW Nordstream
Adamsite metabolites						
Phenarzasinic acid (DM[ox])	4733-19-1	1,400 354 200	7.1 62.1 3.5	17 30* 17*	1.1	CHEMSEA MERCW Nordstream
Sulphur mustard metabolites						
Thiodiglycol sulfoxide (TDG[ox])	3085-45-8	610 3.3	2.1 1.7	1,320* 7.1*		CHEMSEA MERCW
Thiodiglycolic acid	123-93-3	550	1.1	1,137*		CHEMSEA
1,4-Dithiane	505-29-3	45	5.4	55*		CHEMSEA
1,4-Oxathiane	15980-15-1	120	1.6	80*		CHEMSEA
1,4,5-Oxadithiepane	3886-40-6	40	8.7	19	1.1	CHEMSEA
1,2,5-Trithiepane	6576-93-8	35	8.7	3.4	0.5	CHEMSEA
Lewisite metabolites						
Bis(2-chlorovinyl)arsinic acid (L2[ox])	157184-21-9	54.9	2	17*		Nordstream
Propyl bis(2-chlorovinyl)-arsinothioite (L2[SPr])	677355-04-3	70.3	2	0.37*		Nordstream
Organoarsenicals						
Dipropyl phenylarsonodithioite (PDCA[SPr])	1776-69-8	306	26	0.33*		Nordstream
Phenylarsonic acid (PDCA[ox])	98-05-5	1,300 10,833 327	2.2 81.0 2	4 442 599*	2.2 5.2	CHEMSEA MERCW Nordstream
Propyl thioarsenite (TCA[SPr])	5582-57-0	583 39	1.7 13.5	3.6* 0.24*		MERCW Nordstream

| Compound | CAS# | Sediment fraction | Pore water fraction | Source project |

*Calculated from sediment concentration

- Most frequently found→to be tested in chronic test
- Diphenylarsinic- most frequently found in sediment
- Generally low exposure: low detection frequencies and low concentrations, especially in water samples

Literature review: Detected compounds

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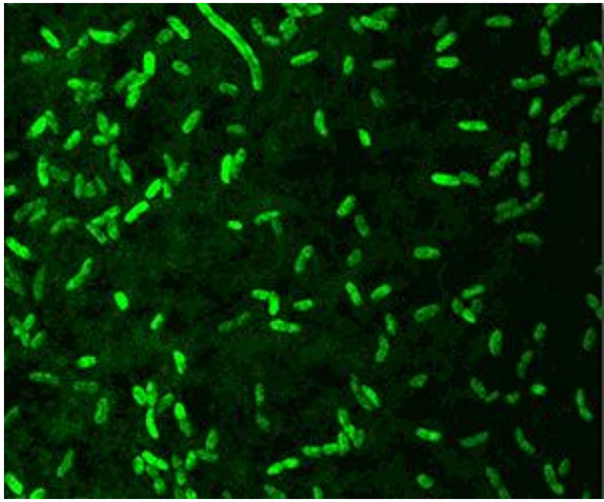
Compound	CAS#	Log K_{OW}	MEASURED Acute LC50 (mg/l)
Parent compounds:			
α-Chloroacetophenone (CAP)	532-27-4	1.93	1.05-1.2 (F) [b]
Triphenylarsine (TPA)	603-32-7	5.97	NA
Clark I metabolites:			
Diphenylarsinic acid (DPA[ox])	4656-80-8	2.8	NA
Adamsite metabolites:			
Phenarzasinic acid (DM[ox])	4733-19-1	2.33	NA
Sulphur mustard metabolites:			
Thiodiglycolic acid	123-93-3	1.16	NA
Thiodiglycol sulfoxide (TDG[ox])	3085-45-8	-2.76	NA
1,4-Dithiane	505-29-3	0.77	23.2 (D), 24.0 (B) [e]
1,4-Oxathiane	15980-15-1	0.53	NA
1,4,5-Oxadithiepane	3886-40-6	1.49	NA
1,2,5-Trithiepane	6576-93-8	2.34	NA
Lewisite metabolites:			
Bis(2-chlorovinyl)arsinic acid (L2[ox])	157184-21-9	1.79	NA
Propyl bis(2-chlorovinyl)-arsinothioite (L2[SPr])	677355-04-3	4.32	NA
Organoarsenicals:			
Dipropyl phenylarsonodithioite PDCA[SPr]	1776-69-8	4.69	NA
Phenylarsonic acid (PDCA[ox])	98-05-5	0.03	>1000 (F) and 710 (F) [d], 800 (F), 900 (F), 420 (F), 600 (F) [b]
Propyl thioarsenite (TCA[SPr])	5582-57-0	3.83	NA
F: fish, D: daphnia, A: algae, B: bacteria, [a] HSDB, [b] USEPA – ECOTOX, [c] Sanderson et. al, 2007 / Munro, 1999, [d] Tsuji et al., 1986 [51], [e] R. Gälli et al., 1994 [52]			

- Samples from the last couples of years narrow down the scope of CWA metabolites
- Predominantly organoarsenicals have been found above the limit of quantification
- Two parent compounds are still found
- Recently metabolites of sulphur mustard has been detected
- Only few compounds have been tested

Methods: Acute toxicity tests

Vibrio fischeri

- Microtox assay with bioluminescent marine bacteria *Vibrio fischeri*
- Endpoint: inhibition of light emission
- Fluorescence measurements 5, 15 and 30 min after exposure
- Assay performed in temperature controlled Microtox M500
- Data collected, stored and analysed on coupled computer



Picture:

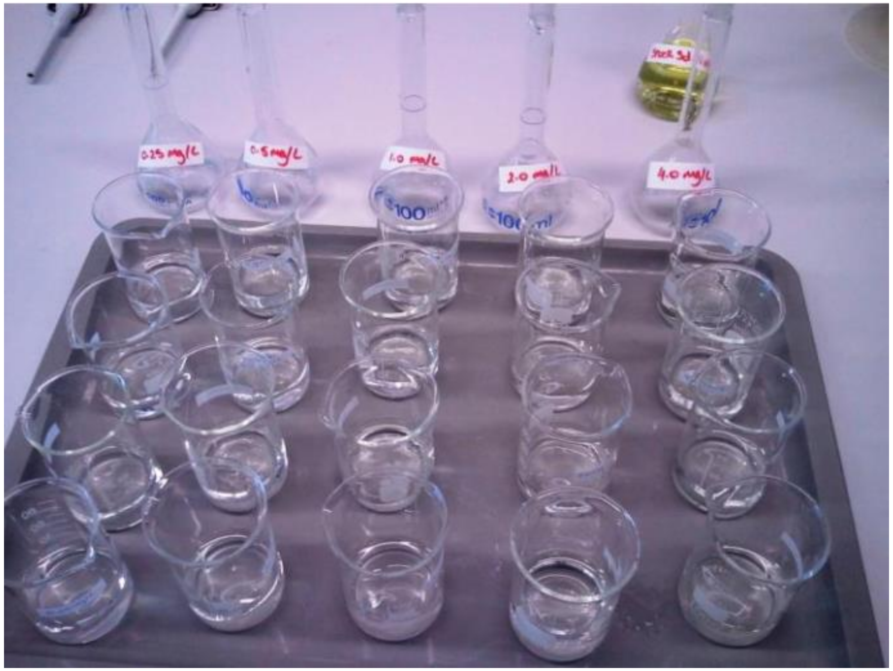
↑ *Vibrio fischeri*

←Setup of Microtox M500 in fume hood, coupled to computer.

Methods: Acute toxicity tests cont’d

Daphnia magna

- 48h day test using crustacean *Daphnia magna*
- Endpoint: immobility/ mortality – range finding test
- Measurements/counting after 24 and 48 hours
- Result: Dose-response curve and EC_{50}



Pictures:

↑ *Daphnia magna*

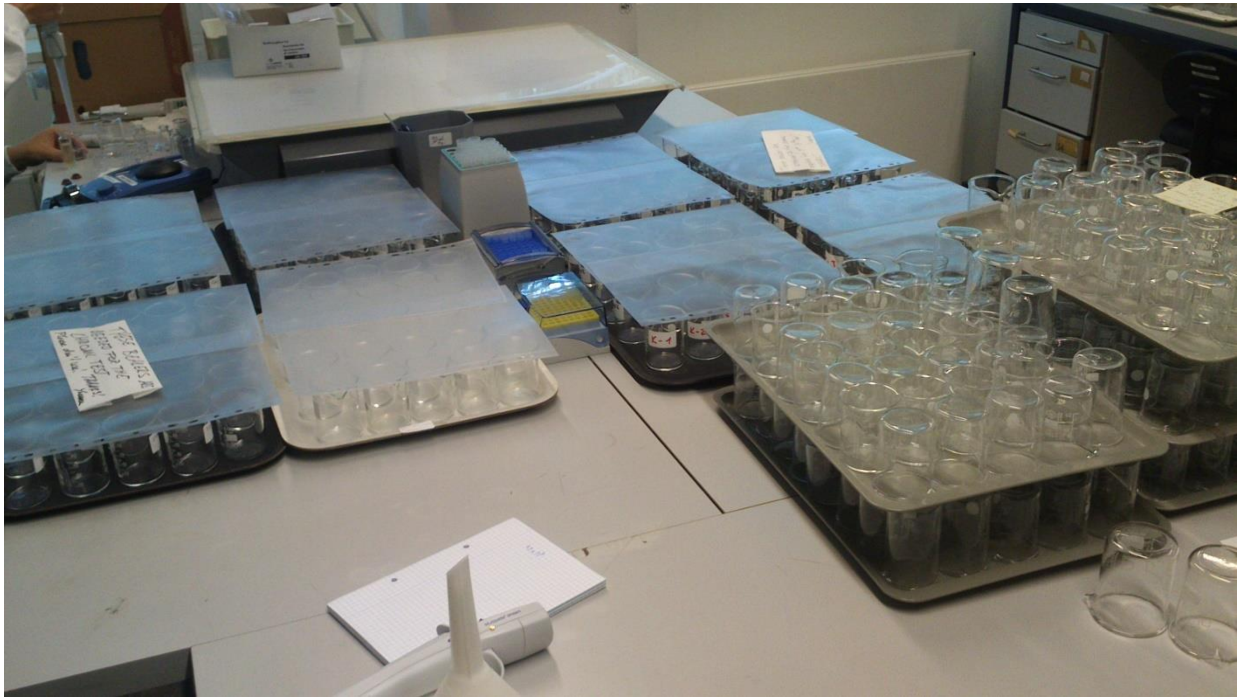
←Beakers needed for one acute test.

Each board has 30-35 beakers.

Methods: Chronic toxicity tests

Daphnia magna

- 21 day test using crustacean *Daphnia magna*
- Endpoint: inhibition of reproduction and mortality
- Measurements/counting every day
- Change of media every 2.-3. day
- Samples taken for chemical analysis once a week



Pictures:

↑ *Daphnia magna*

←Beakers needed for 14 days of testing.

Each board has 30-35 beakers.

Results: Acute toxicity (*V.fischeri*)

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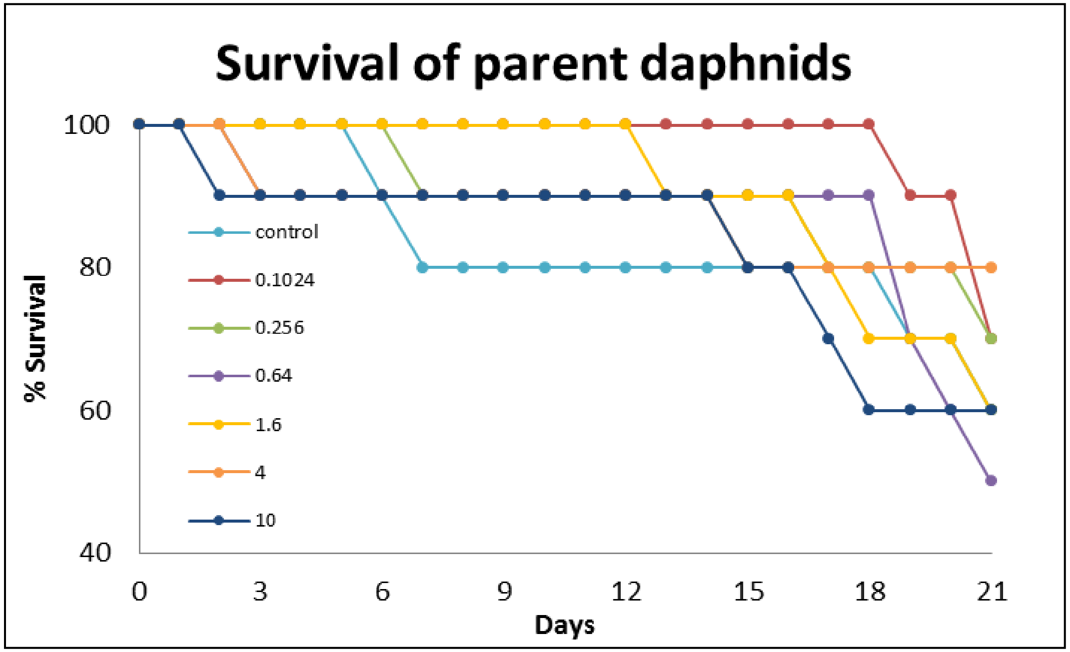
Compound	CAS#	V.fischeri LC50 (mg/l)	Measured C_W or PEC [µg/L]
Parent compounds			
α-Chloroacetophenone (CAP)	532-27-4	0.0112	0.92[c]
Triphenylarsine (TPA)	603-32-7	>50[a]	68
Clark I metabolites			
Diphenylarsinic acid (DPA[ox])	4656-80-8	124	1,538
Adamsite metabolites			
Phenarzasinic acid (DM[ox])	4733-19-1	Insoluble: <10[b]	17
Sulphur mustard metabolites			
Thiodiglycol sulfoxide (TDG[ox])	3085-45-8	>450	1,320[c]
Thiodiglycolic acid	123-93-3	22.5	1,137[c]
1,4-Dithiane	505-29-3	Insoluble: <10[b]	55[c]
1,4-Oxathiane	15980-15-1	47.4	80[c]
1,4,5-Oxadithiepane	3886-40-6	1.70[a]	19
1,2,5-Trithiepane	6576-93-8	Not tested	3.4
Lewisite metabolites			
Bis(2-chlorovinyl)arsinic acid (L2[ox])	157184-21-9	Not tested	17[c]
Propyl bis(2-chlorovinyl)-arsinothioite (L2[SPr])	677355-04-3	Not tested	0.37[c]
Organoarsenicals			
Dipropyl phenylarsonodithioite (PDCA[SPr])	1776-69-8	Not tested	0.33[c]
Phenylarsonic acid (PDCA[ox])	98-05-5	97.1	442
Propyl thioarsenite (TCA[SPr])	5582-57-0	Not tested	3.6[c]

[a] Tested using solvent, [b] Solubility value, [c] Derived from measured sediment concentration

- 8 out of 15 compounds tested
- EC50 obtained for 6 compounds
- Most toxic compounds are chloroacetophenone (EC50=0.0112 mg/L), 1,4,5-oxadithipane (EC50=1.70 mg/L) and thiodiglycolic acid (EC50=22.5 mg/L)

Results: Chronic tests - *D. magna*

- Compound: Diphenylarsinic acid
- Validity met until day 19 where control animals survival was insufficient
- Daphnia sensitive to physical impact



Results: Chronic tests - *D. magna* cont'd

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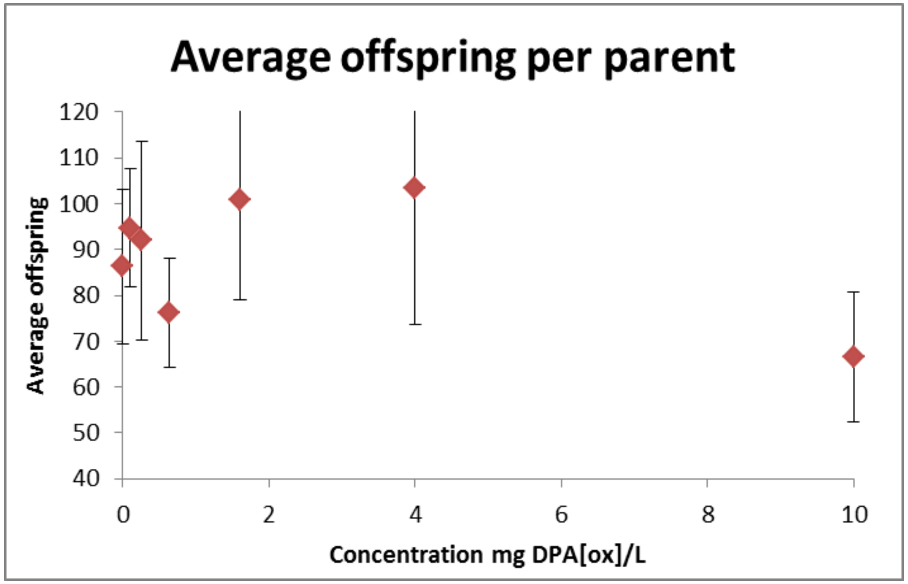
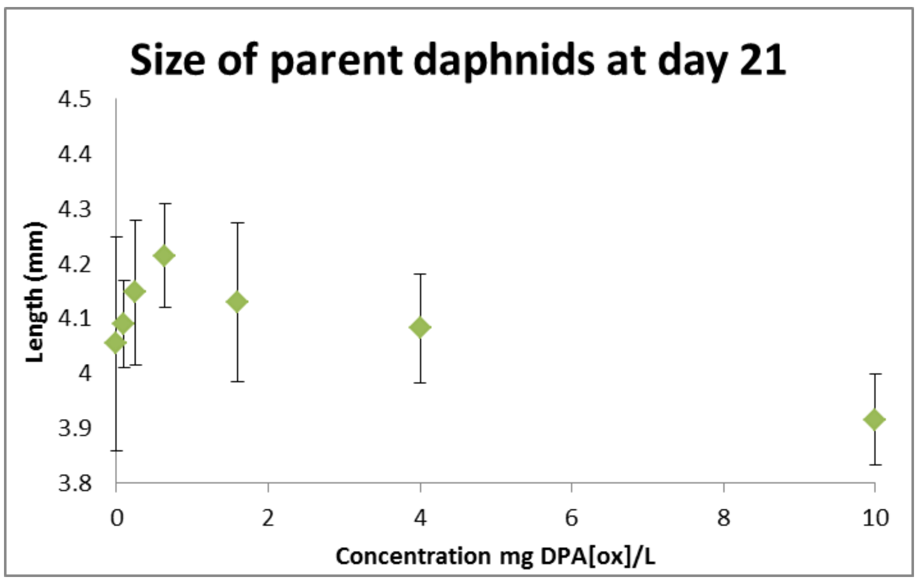
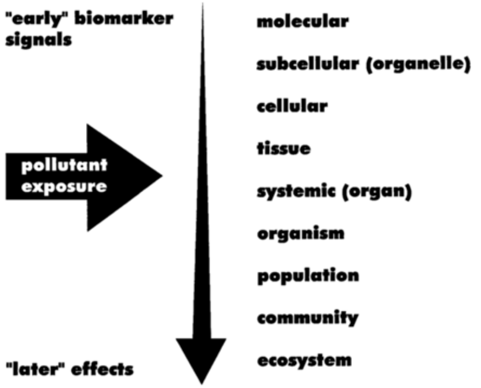


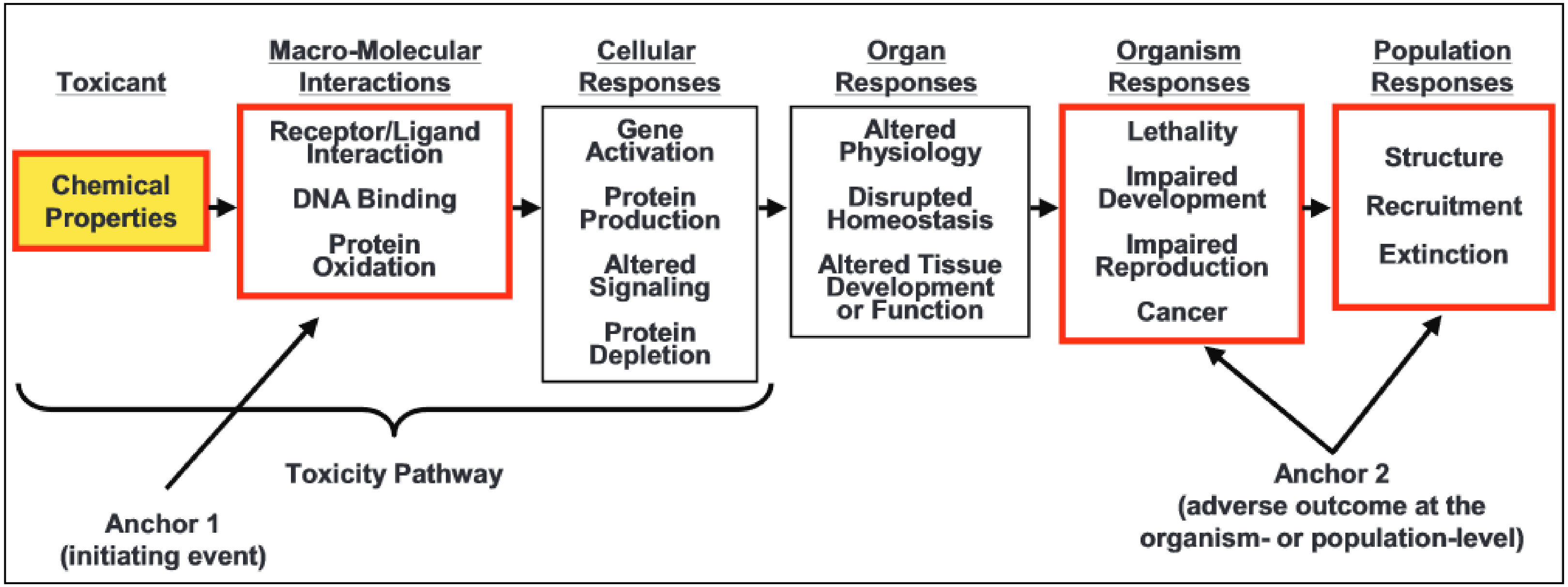


- Compound: Diphenylarsinic acid
- No significant difference from control and highest concentration in regards to average offspring or size of parent daphnia
- Highest concentration should be increased in a new test

Adverse Outcome Pathways

- Small fraction of outcome identified
- Short-term: Narcotic MoA . Long-term: ?
- Uncertainty increases at population level
- Linkage between chemistry and biology
- Interdisciplinary approach needed





Source: Ankley et al., 2010

Results: Toxicant-gene interactions

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Genes affected by detected CWAs

Genes present in fish?

ctd™ *Illuminating how chemicals affect human health.*
Comparative Toxicogenomics Database

Genes expression probable in fish?

=

Hypothesis +

Sub-lethal effects to look for in future tests

Compound	Interaction (human)	Diseases (human)	Likelihood in fish*	Inference score
Phenylarsonic acid PDCA[ox]	increased expression of HMOX1 mRNA	Gastroparesis	Likely	5.96
		Adrenoleukodystrophy	Likely	5.84
		Systemic mastocytosis	Likely	5.84
α-Chloroacetophenone CAP	increased expression of CALCA protein	Migraine without Aura	Less likely	12.98
	increased expression of CYB5A protein	Neurogenic inflammation	Likely	12.37
	increased expression of TAC1 protein	Arthritis	Less likely	10.96
	increased expression of TAC2 protein			
Diphenylarsinic acid DPA[ox]	increased expression of BCL2 mRNA	-		NA
	increased activity of CASP3 protein			
	increased cleavage of GCLC protein			
	increased expression of CCT2			
	increased expression of CYP1B1 mRNA			
	decreased expression of CYP3A2 mRNA			
	decreased expression of DLG1 protein			
	increased expression of GCLC protein			
	decreases expression and increases degradation of GLS			
	increased expression of HMOX1 protein			
	effect on the localization and increased activity of NFE2L2 protein			
	increased expression of PTGS2 mRNA			
	increased expression of RPLP0 protein			

*Personal estimation

- 3 compounds have documented interactions with 18 genes present in fish.
- Different human diseased related to expression of these genes (digestion; nerve; immuno; inflamation) – in fish: growth impairment
- Inference scores are relatively low

Conclusion

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- Acute EC50s obtained for 6 out of 15 detected compounds
  - Remaining compounds will be assessed this winter
- Chronic marine risk cannot be ruled out.
  - Further research both on exposure and toxicity needs elucidation
- Chronic test will be conducted and linked to the fish health observations in MODUM.
  - Possible chronic effects – growth inhibition

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Thank you for your attention!

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