



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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Introduction

- › 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 is the environmental toxicity of CWAs found?



Introduction cont'd

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

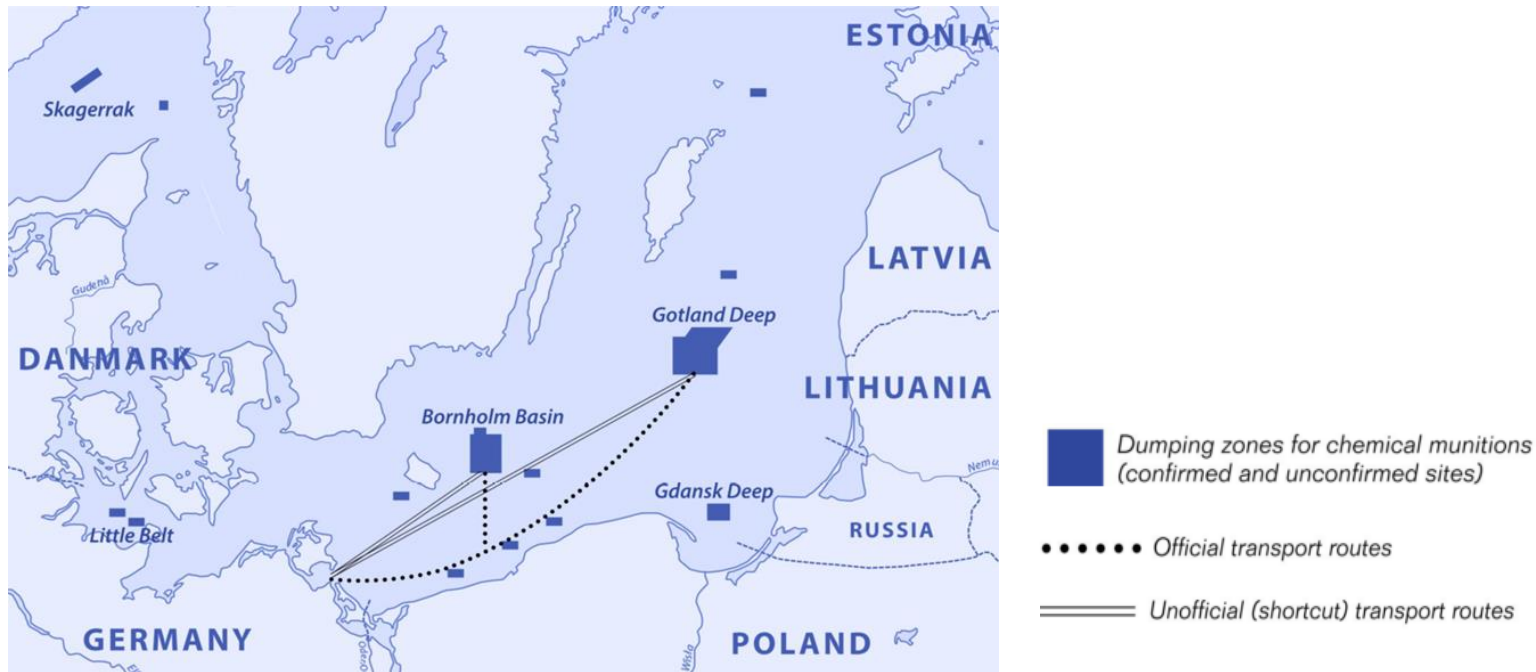


Table 1: Confirmed dumped CWAs in the Bornholm dumpsite east of Bornholm. It is the largest dumpsite in the Baltic Sea in terms of the amount of dumped munitions. Source: HELCOM, 1994.

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

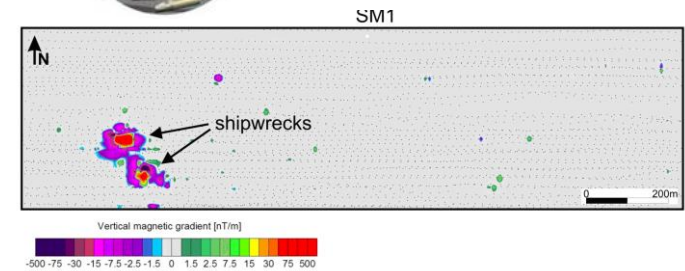
¹Blistering agent, ²Organoarsenic blistering agent, ³Additive, ⁴Arsine oil constituent, ⁵Riot control agent,

⁶Blood 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



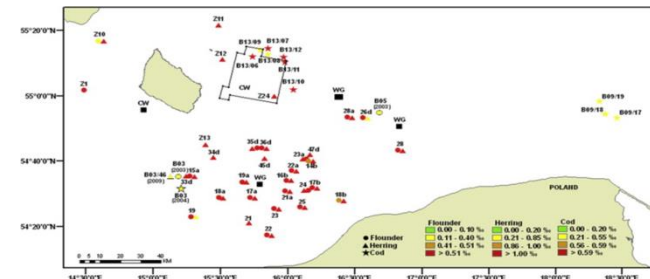
CHEMSEA (2011-2014)

Chemical Munitions Search and Assessment

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



CHEMSEA
CHEMICAL MUNITIONS
SEARCH & ASSESSMENT



Nord Stream pipeline construction (2008-2012)

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



MODUM (2014-2016)

- Only a few samples collected – focus on method development
- ROV and AUV used – collect samples from an object



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Effects assessment

PBT screening profile of chemical warfare agents (CWAs)

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Abstract

Chemical warfare agents (CWA) 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 (QSAR) 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 (QSAR 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 organophosphorus 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).

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Keywords: Chemical weapon agents; QSAR; Environmental toxicity; EU TGD; Marine toxicity

> Lack of CWA ecotoxicological data

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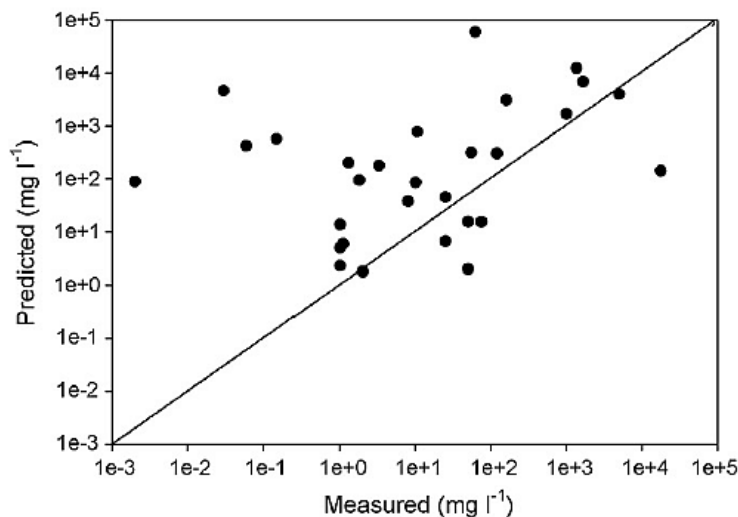


Fig. 1. Measured vs. predicted LC50 values—species specific (mg l^{-1}).

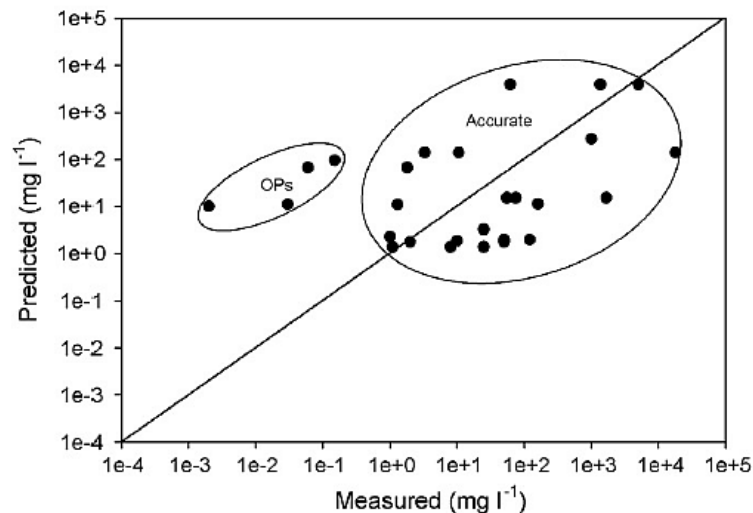


Fig. 2. Measured vs. predicted LC50—not species specific (lowest predicted) (mg l^{-1}).

> Use QSAR model – since mostly found organoarsenicals we can use inorganic arsenic as a conservative surrogate for extrapolation

Effects assessment, cont'd

Screening level fish community risk assessment of chemical warfare agents in the Baltic Sea

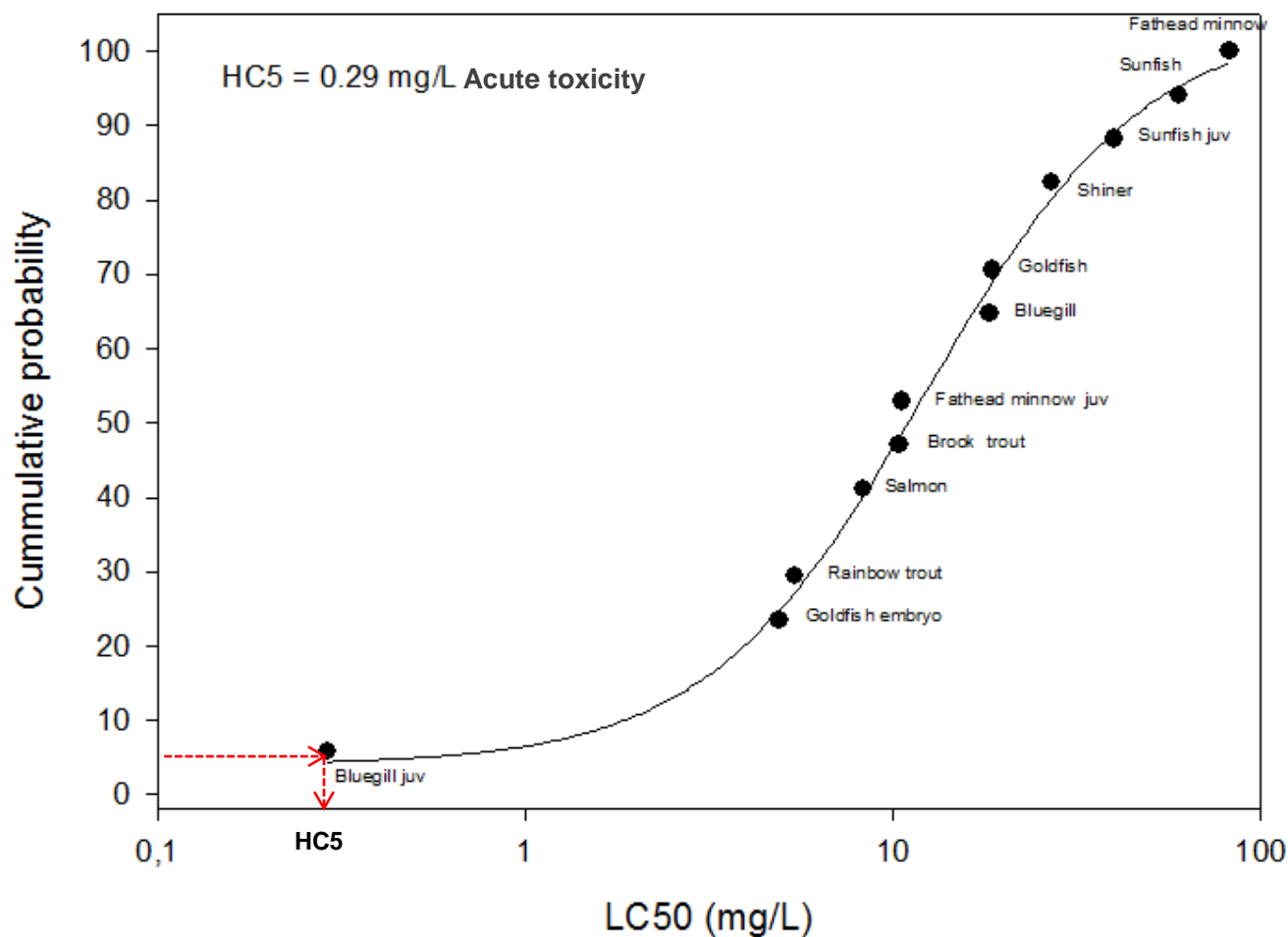
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Organoarsenic fish community SSD



Mustard gas fish community HC5 = 0.69 mg/L

[Monro et 1999; USEPA WEB ICE model extrapolated Bluegill sunfish NOEC = 2 mg/L]

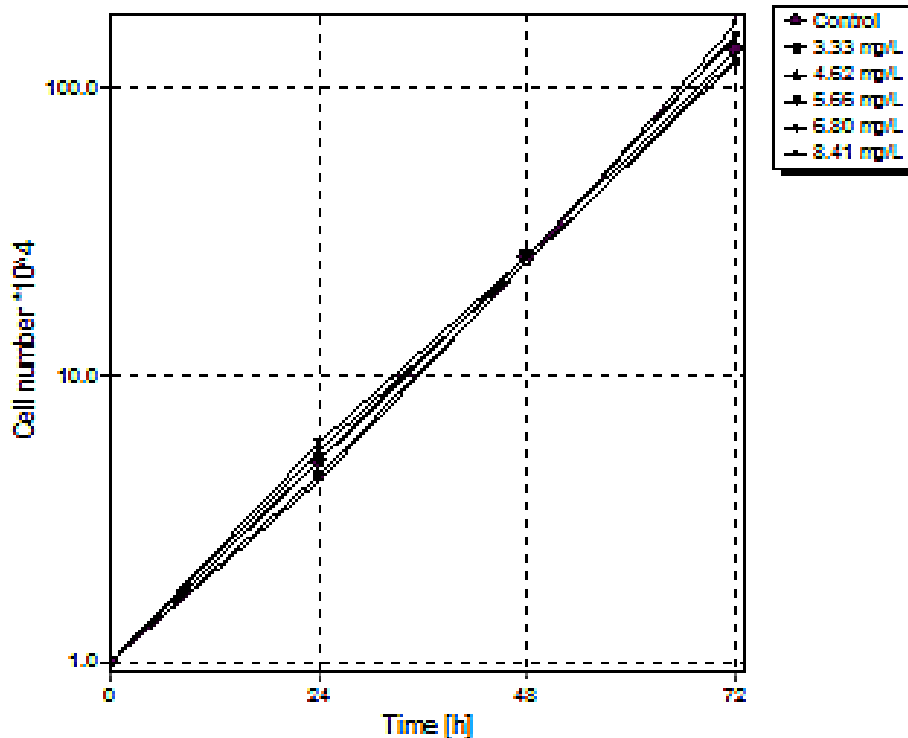
Common Name	Scientific	Estimated Toxicity	95% Confidence Intervals ug/L	Surrogate	Degrees of Freedom (N-2)	R2	p-value	Mean Square Error	Cross-validation Success (%)	Taxonomic Distance	Slope	Intercept
Atlantic salmon	<i>Salmo salar</i>	1142.57	703.59 - 1855.42	Bluegill (Lepomis macrochirus)	9	0.96	0.0000	0.08	100.00	4	1.12	-0.64
Brook trout	<i>Salvelinus fontinalis</i>	1070.14	609.64 - 1878.50	Bluegill (Lepomis macrochirus)	16	0.88	0.0000	0.20	88.88	4	1.05	-0.44
Brown trout	<i>Salmo trutta</i>	1320.28	725.92 - 2401.28	Bluegill (Lepomis macrochirus)	12	0.94	0.0000	0.12	100.00	4	1.06	-0.39
Channel catfish	<i>Ictalurus punctatus</i>	3192.69	2171.92 - 4693.20	Bluegill (Lepomis macrochirus)	71	0.75	0.0000	0.44	76.71	4	0.79	0.87
Chinook salmon	<i>Oncorhynchus tshawytscha</i>	557.05	169.06 - 1835.42	Bluegill (Lepomis macrochirus)	4	0.90	0.0035	0.20	83.33	4	1.34	-1.69
Coho salmon	<i>Oncorhynchus kisutch</i>	1614.65	812.40 - 3209.11	Bluegill (Lepomis macrochirus)	11	0.91	0.0000	0.15	92.30	4	0.98	-0.04
Common carp	<i>Cyprinus carpio</i>	3207.82	1483.08 - 6938.33	Bluegill (Lepomis macrochirus)	17	0.82	0.0000	0.37	84.21	4	0.85	0.67
Fathead minnow	<i>Pimephales promelas</i>	2894.85	2011.69 - 4165.72	Bluegill (Lepomis macrochirus)	66	0.79	0.0000	0.43	75.00	4	0.84	0.66
Goldfish	<i>Carassius auratus</i>	4299.28	2180.67 - 8476.20	Bluegill (Lepomis macrochirus)	21	0.80	0.0000	0.42	86.95	4	0.81	0.93
Guppy	<i>Poecilia reticulata</i>	3483.19	1488.44 - 8151.21	Bluegill (Lepomis macrochirus)	15	0.83	0.0000	0.44	76.47	4	0.80	0.89
Lake trout	<i>Salvelinus namaycush</i>	863.02	495.58 - 1502.89	Bluegill (Lepomis macrochirus)	20	0.70	0.0000	0.27	86.36	4	0.68	0.68
Rainbow trout	<i>Oncorhynchus mykiss</i>	1486.67	1317.93 - 1677.01	Bluegill (Lepomis macrochirus)	307	0.88	0.0000	0.21	90.61	4	0.93	0.08
Sheepshead minnow	<i>Cyprinodon variegatus</i>	2560.64	1814.03 - 3614.53	Bluegill (Lepomis macrochirus)	70	0.71	0.0000	0.39	86.11	4	0.80	0.75
White sucker	<i>Catostomus commersoni</i>	1406.96	327.90 - 6036.99	Bluegill (Lepomis macrochirus)	2	0.97	0.0141	0.04	100.00	4	1.16	-0.68



TDG NOEC
42 day =
1000 mg/L
(not toxic)

Mustard gas cyclic dissipation products NOEC? AARHUS UNIVERSITET

Preliminary result *Raphidocelis subcapitata* algae growth and yield 72hr for 1-Oxa-4,5-dithiepane]



We use 1-Oxa-4,5-dithiepane as representative of all the products. Algae 72hr NOEC = 8 mg/L

Daphnia magna 21-d test is ongoing – preliminary range finding results approx. 1 mg/L

Yields an assessment factor of 500 (EU TGD, 2003) to the marine PNEC

Measured sediment and measured + predicted porewater concentrations

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
Clark I metabolites						
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

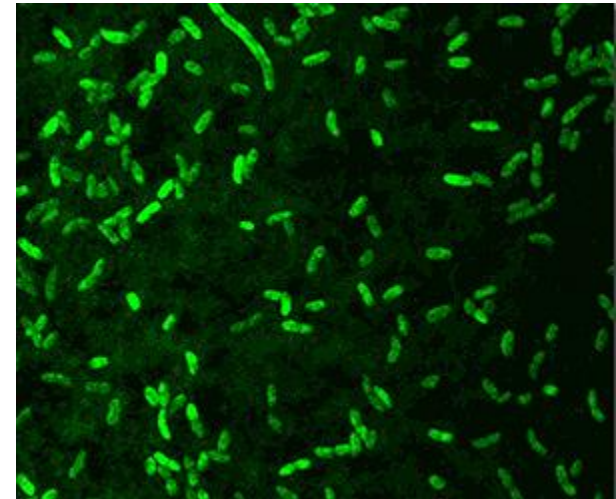
*Calculated from sediment concentration

- Increasing analytical capabilities → more finds
- Until recently predominantly organoarsenicals have been found above the limit of quantification
- Parent compounds are still found
- Recently metabolites of sulphur mustard has been detected
- Only few compounds have been tested for ecotoxicity
- New data from Nord Stream II will emerge

Methods: Acute toxicity tests

Allivibrio fischeri

- Microtox assay with bioluminescent marine bacteria *Allivibrio fischeri* ISO standardized test
- 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:

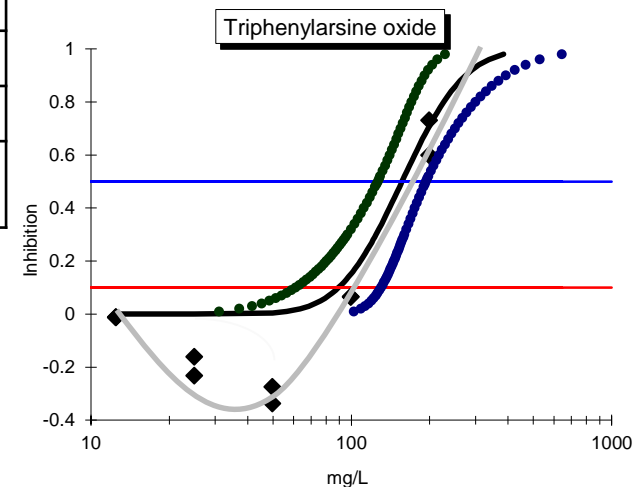
↑ *Allivibrio fischeri*

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

Results: Acute toxicity (*A. fischeri*)

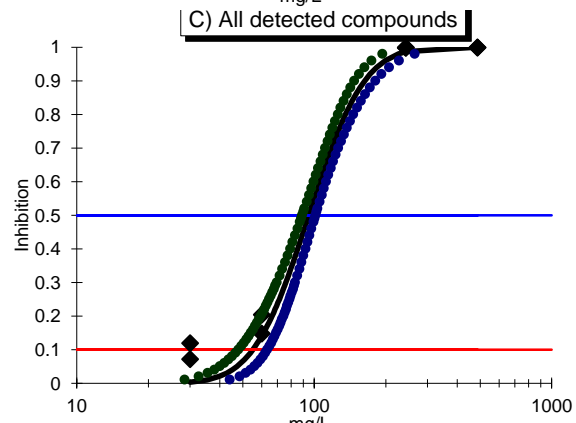
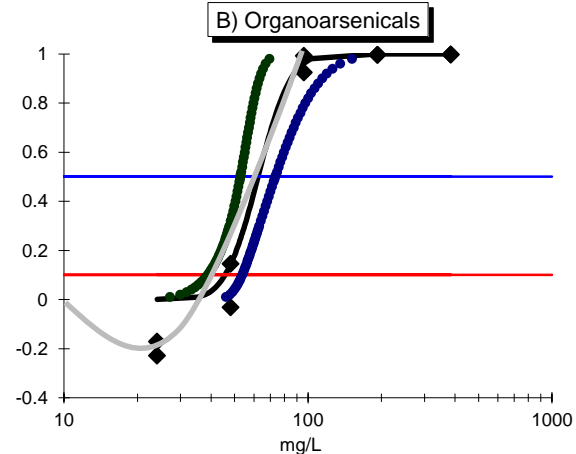
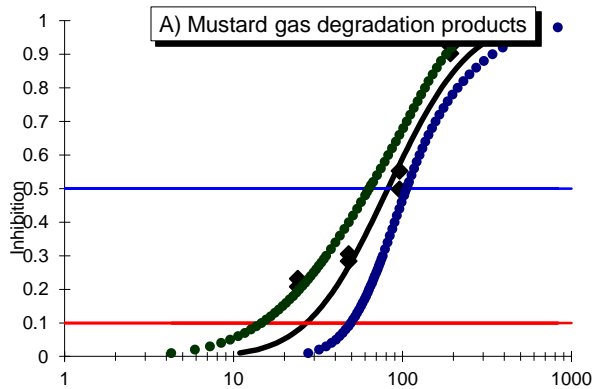
Compound	CAS#	LogKow	Water Solubility [mg/L]	EC50 <i>A. fischeri</i> (95% conf.int.) [mg/L]
Triphenylarsine ¹	603-32-7	5.97	0.12	>200*
Triphenylarsine oxide	1153-05-5	5.97	0.07	155* (124-194)
α -chloroacetophenone ¹	532-27-4	1.93	1,635	0.0112 (0.0086-0.0145)
1,4-dithiane ²	505-29-3	0.77	3.000	9.97* (8.36-11.90)
1,4-oxathiane ²	15980-15-1	0.53	39,880	47.4 (42.1-53.3)
1,4,5-oxadithiepane ²	3886-40-6	1.49	16,277	1.70* (1.46-1.99)
1,2,5-trithiepane ²	6576-93-8	2.34	769	1.17* (0.92-1.50)
Thiodiglycol sulfoxide ²	3085-45-8	-2.76	1,000,000	>74250
Thiodiglycolic acid ²	123-93-3	1.16	400,000	22.5 (21.3-23.8)
Phenylarsonic acid ³	98-05-5	0.03	1,000,000	97.1 (91.5-103)
Diphenylarsinic acid ⁴	4656-80-8	2.8	1,629	124 (118-137)
Phenarzasinic acid ⁵	4733-19-1	2.33	3,338	5.33* (5.02-5.67)
2-chlorovinylarsonic acid ⁶	64038-44-4	-0.472	1,296*10 ⁵	0.0312* (0.0284-0.0343)
Bis(2-chlorovinyl)arsinic acid ⁶	157184-21-9	1.79	38,204	Not tested (Insoluble)

- 13 out of 14 compounds tested with EC50s obtained
- Most toxic compounds are chloroacetophenone (EC50=0.0112 mg/L), 1,4,5-oxadithiepane (EC50=1.70 mg/L) and thiodiglycolic acid (EC50=22.5 mg/L)
- Hormetic effect observed for TPAox (grey line)



¹Parent compound, ²Sulphur mustard metabolite, ³Phenyldichloroarsine (PDCA) metabolite, ⁴Clark I metabolite, ⁵Adamsite metabolite, ⁶Lewisite metabolite *Solvent used (acetonitrile)

Mixture toxicity

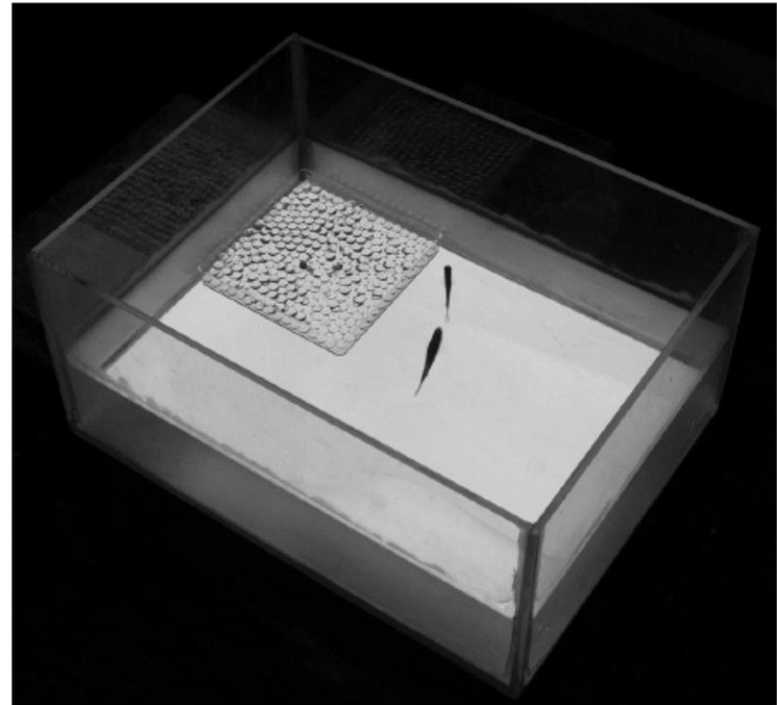
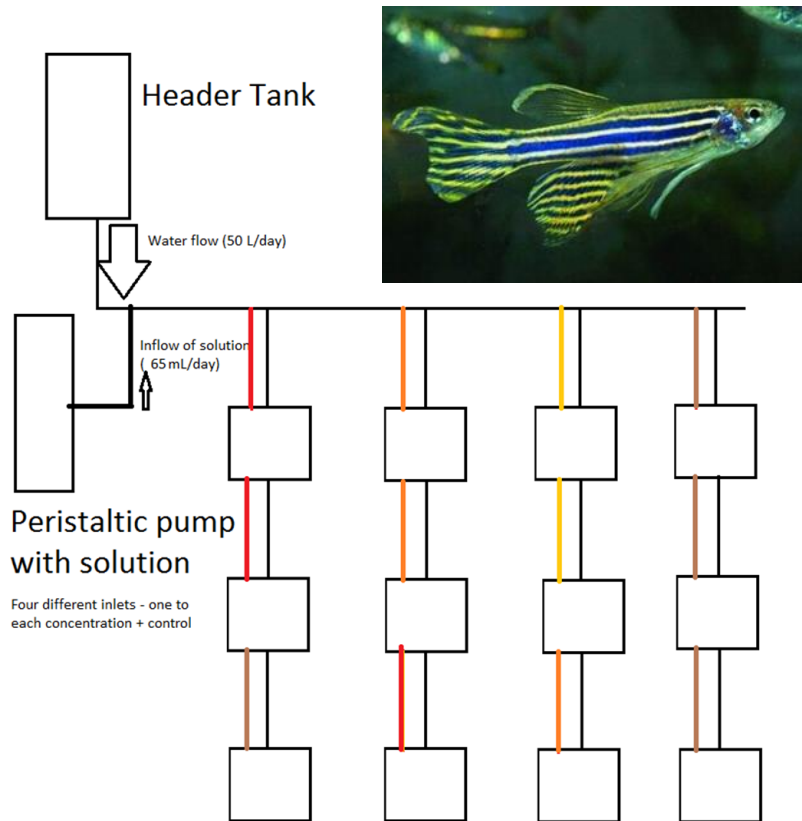


Mixture	CAS #	EC ₅₀ predicted [mg/L]	EC ₅₀ found (95% conf.int.) [mg/L]
Sulphur mustard gas bomb	505-29-3, 15980-15-1, 3886-40-6, 6576-93-8, 3085-45-8, 123-93-3	16.5	82.0 (63.6-106)
Three most toxic Sulphur mustard gas compounds	505-29-3, 3886-40-6, 6576-93-8	4.21	9.71 (7.80 – 12.1)
Organoarsenic mixture (except TPA, L1 [ox]) ^a	98-05-5, 4656-80-8, 4733-19-1, 1153-05-5	95.4	62.4 (52.9 – 73.6)
All tested compounds	505-29-3, 15980-15-1, 3886-40-6, 6576-93-8, 3085-45-8, 123-93-3, 98-05-5, 4656-80-8, 4733-19-1, 1153-05-5, 532-27-4	61.6	94.8 (88.9 – 101)

No synergistic effects identified – suggesting a similar non-specific mode of action

Hormetic effect was found for the organoarsenic CWA residues (B)

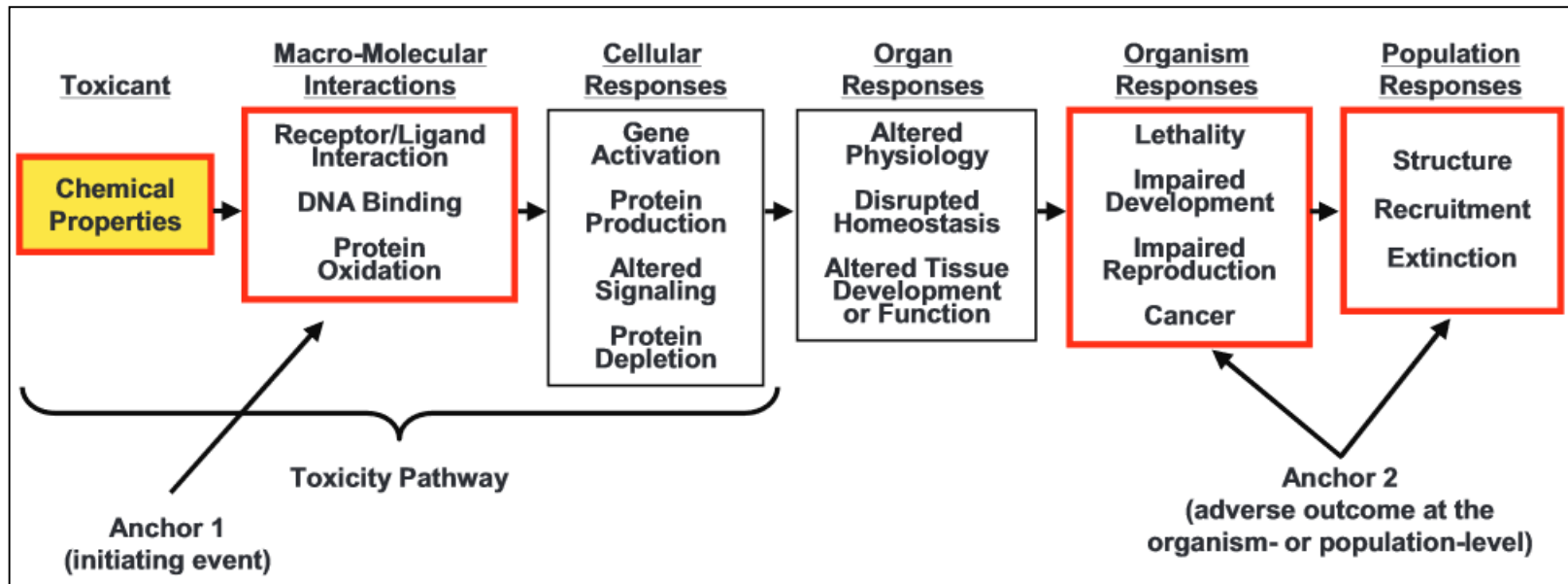
Locomotion test with zebra fish (2 weeks)



Assess if locomotion and behavior is impacted - as an indicator of overall health and fitness

Conclusions

- › The short term/acute toxicity MoA is non specific, additive
- › TPAox and mixed organoarsenicals show hormetic effects in Microtox tests
- › Uncertainty about chronic and long term toxicity
- › Uncertainty regarding regulatory relevance of the toxicity and risk



Source: Ankley et al., 2010

Methods: Gene-toxicant interactions

- › *In silico* investigation comparing human toxicogenomics with fish (*Danio rerio*) genomics



illuminating how chemicals affect human health.

Comparative Toxicogenomics Database

Genes affected by detected CWAs



National Center for Biotechnology Information

Genes present in fish?



Zebrafish Gene Collection



Genes expression probable in fish?



Hypothesis - sub-lethal effects to look for in future tests?

Conclusions Toxicant-gene interactions

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

- › Cytotoxic MoA knowledge can be transferred (e.g. DNA acylation)
- › 3 compounds have documented interactions with 18 genes present in fish.
- › Different human diseases related to expression of these genes (digestion; nerve; immuno; inflammation) – in fish → growth impairment

Thank you for your attention!



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