



Biosensors for Aqueous Toxicants: a Novel DNA-Based System Compared with *C. dubia*, Acute Microtox[®] and Sub-Mitochondrial Particle (SMP) Assays



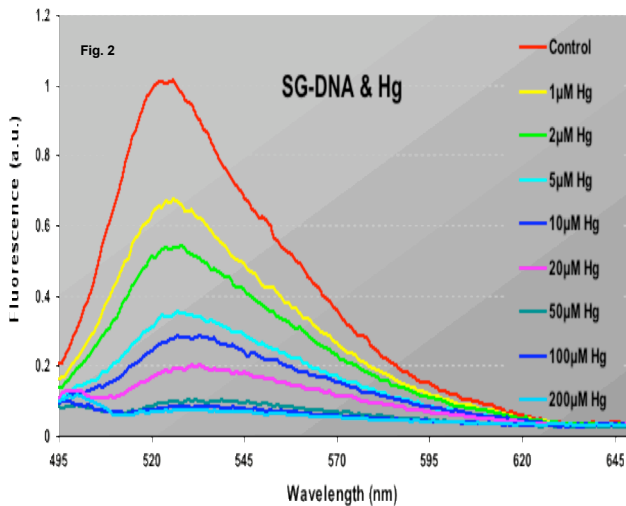
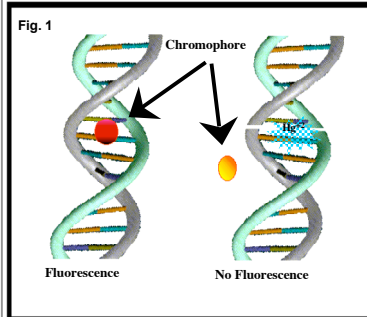
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Introduction

Several bioassays can assess toxic potential in environmental samples, some of them with high sensitivity. They provide standardized measurements of toxicity that help to manage the risks that those pollutants represent for the environment and the public health.

We have developed novel biosensing technology based on fluorescent ligand/dye and DNA. The theoretical limits of detection in this assays are determined by the fluorescence detecting equipment, and the affinity of the dye for DNA. DNA, being a negatively charged bio-polymer, can interact with a wide range of chemical cations. Some metal ions can produce single and double strand breaks in the DNA and alter its conformation.

Our assay takes advantage of the ability of heavy metals ions to displace the fluorescent dye from DNA producing a free dye that is hardly fluorescent. Fig. 1 illustrates this concept.

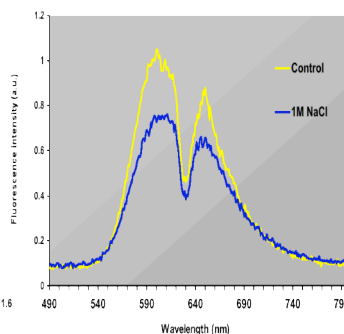
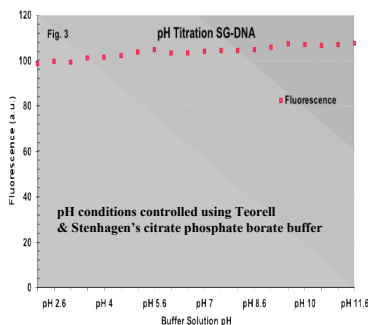


Method

DNA Assay The DNA assay was performed using calf thymus DNA (Sigma-Aldrich (cat# D 1501)). The samples were filtered through a 0.45 µm Millipore Millex[®]-HN Syringe Driven Filter (cat# SLNH-013-NL) to eliminate any particle interference with the fluorescence reading. The assay conditions were: calf thymus DNA (0.1 µg/ml); 1/40,000 dilution for SYBR Green I (Molecular Probes, OR); 30 mM Tris pH 8.8 in a total sample volume of 3 ml. Standardized mercuric chloride (Sigma, cat# M 1136) was serially diluted from a 1 M stock. Fluorescence was measured using a Shimadzu RF5000 fluorometer (Shimadzu Kabushikikaisha, Naniigawa, Japan) at 23 °C.

Sub-Mitochondrial Particle Assay Sub-mitochondrial particles (SMPs) derived from beef heart were purchased from MitoScan Corp., Madison, USA. A modified spectrophotometric method (Blondin et al., 1987) measured the rate of NAD reduction in a Reverse Electron Transfer (RET) test at 340 nm (Oakes and Pollak, 1999). The effluent samples were tested at 70% v/v and the results were compared with a negative control (buffer) solution.

Microtox[®] Basic Acute Test This test was performed as recommended in the Microbics manual (1995 'Microtox[®] Acute Toxicity Basic Test Procedures'). All samples were adjusted to a salinity of 20‰ prior to Microtox[®] tests, by dissolving a calculated weight of NaCl into the sample. The test temperature was 15°C and readings of bacterial light output were taken after 5 min exposure.



Methods (continued)

Ceriodaphnia dubia Acute Test

The *C. dubia* acute test is based on procedures published by the USEPA (2002). Ours differed from this guideline in that an Australian cladoceran, *Ceriodaphnia cf dubia* was used, and animals were cultured using only algae as food.

Results and Conclusions

Table 1 Assay results for all samples examined by four different toxicity assays.

Table 2 Shows a simplified assessment of toxicity obtain from the analysis of the results data.

Sample	DNA Assay Norm. reading	<i>C.dubia</i> 48 hr EC50	SMP Norm. reading	Acute Microtox 5 min EC50
50µM HgCl2	0.33	0	0.69	0.69
25µM HgCl2	0.63	0	0.54	0
48H2O	1.00	no immobilisation	1.00	0
3049	0.81	no immobilisation	0.83	0
3050	0.62	34.6	1.00	0
3051	0.36	34.6	0.96	0
3052	0.45	54.8	1.02	0
3053	0.38	40% at 100% sample	0.95	0
3054	0.53	15.1	1.00	0
3055	0.14	0.55	0.87	0
3056	0.71	17.3	0.97	0
3057	0.41	54.8	0.94	0
3058	0.45	no immobilisation	1.06	0
3059	0.38	100	0	0
3060	0.61	54.8	0.95	0
3061	0.62	27.5	0.95	0
3062	0.24	81.8	0	0
3063	0.13	no immobilisation	1.05	0
3064	0.62	no immobilisation	1.06	0
3065	0.77	no immobilisation	1.06	0
3066	0.56	30% at 100% sample	1.05	0
3067	0.60	2.4	0.00	112.70
3068	0.13	100	0.97	0
3069	1.01	no immobilisation	0.89	0
3070	0.62	30.8	1.00	0
3071	0.55	41.2	1.00	0
3072	0.57	54.8	1.00	0
3073	0.42	no immobilisation	1.01	0
3074	0.82	17.3	0.96	0
3075	0.22	6.2	0.00	117.30
3076	0.79	40% at 100% sample	0.96	0
3077	0.82	13.1	0.94	0
3078	1.00	3.2	0.94	229.40
3079	0.92	3.8	0.99	0
3080	1.00	3.2	0.96	0
3081	1.01	3.8	0.35	27.21
3082	1.02	7.1	0.81	84.94
3083	0.58	7.4	1.09	0
3084	0.47	4.4	0.00	98.49
3085	0.22	no immobilisation	0.03	0
3086	0.22	56.6	0.93	0
3087	0.84	no immobilisation	0.88	0
3088	0.70	no immobilisation	0	0

This preliminary study indicates the novel DNA toxicity assay constitutes a valid platform for detecting toxicity. In addition, the comparative study suggests a reasonably good level of agreement with other standard toxicity assays, such as the *C. dubia* 48 hr acute toxicity assay. It also shows that this novel DNA toxicity assay has a higher sensitivity than other well known acute toxicity assays, such as Acute Microtox[®] (5 min). Accordingly, further research is needed as to establish a better understanding of the spectrum of detectable toxicants and sensitivity ranges of this novel DNA bioassay. Non-the-less, this new bioassay could represent a significant improvement for environmental monitoring and toxicity assessing tasks providing simple, sturdy, highly sensitive, ultra-fast and inexpensive means to carry out toxicity screening tasks in the field.

Sample	DNA Assay Norm. reading	<i>C.dubia</i> 48 hr EC50	SMP Norm. reading	Microtox 5 min EC50
50µM HgCl2	T	0	VT	0
25µM HgCl2	T	0	0	0
0	NT	NT	NT	NT
48H2O	NT	NT	NT	NT
3049	NT	NT	NT	NT
3050	T	T	NT	NT
3051	T	T	NT	NT
3052	T	T	NT	NT
3053	T	T	NT	NT
3054	T	T	NT	NT
3055	T	VT	NT	NT
3056	T	T	NT	NT
3057	T	T	NT	NT
3058	T	NT	NT	NT
3059	T	T	NT	NT
3060	T	T	NT	NT
3061	T	T	NT	NT
3062	0	T	NT	NT
3063	T	NT	NT	NT
3064	T	NT	NT	NT
3065	T	T	NT	NT
3066	T	T	NT	NT
3067	T	VT	T	T
3068	T	VT	NT	NT
3069	NT	NT	NT	NT
3070	NT	NT	NT	NT
3071	T	T	NT	NT
3072	T	T	NT	NT
3073	T	NT	NT	NT
3074	NT	T	NT	NT
3075	T	VT	VT	T
3076	T	T	NT	NT
3077	NT	T	NT	NT
3078	NT	VT	NT	NT
3079	NT	VT	NT	NT
3080	NT	VT	NT	NT
3081	NT	VT	T	T
3082	NT	VT	NT	T
3083	T	VT	NT	NT
3084	VT	VT	T	T
3085	T	NT	VT	NT
3086	T	T	NT	NT
3087	NT	NT	NT	NT

NT=non-toxic; T=toxic; VT=very toxic

References

Blondin GA, Knobeloch LM, Read HW, et al. (1987) "Mammalian mitochondria as in vitro monitors of water quality". Bull. Environ. Contam. Toxicol. 38: 467-474.
Oakes DJ, Pollak JK. (1999). "Effects of a herbicide formulation, Tordon 75D, and its individual components on the oxidative functions of mitochondria." Toxicology 136: 41-52.
The IP disclosed in this poster is covered by a global patent (pending).