an animal alternative bioassay for toxicity measures for water soluble samples.

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ABSTRACT

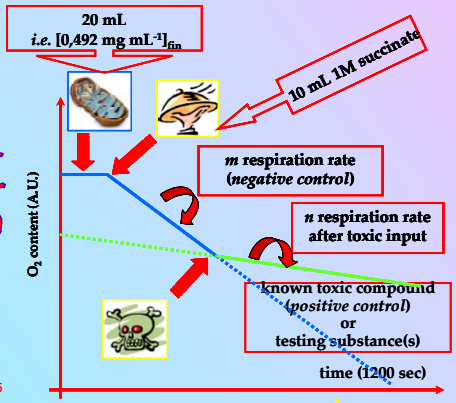
Several methods have been proposed, using responses of whole organisms. The problem, however, is not only strictly scientific, but also involves cost, resources and time. For example, assay with organisms require expensive testing facilities and long operational times are necessary for toxicity measurements. In order to evaluate potential compound toxicity (acute and sub-chronic), we standardized a bioassay using mitochondria of beef hearth, and their applicability and sensitivity was verified. In respect to other based on mitochondria tests, this bioassay (called FM22) showed unquestionable advantages: i) to freeze mitochondria at -22 °C instead of the classical -80 °C, ii) to perform a very big quantity of biological test using always the same mitochondria pool (avoiding differences from age, sex, or health status depending on different organisms); iii) to identify quickly a tested compounds IC50, easily comparable. FM22 end point is the inhibition of mitochondrial respiratory chain and this event is quantified by oxygen monitoring. The oxygen consumption was measured by a Clark electrode that was interfaced to a PC to collect test analysis data (1200 in 20 min run). A piecewise regression, through an Excel® Macro, identified the break point in the oxygen consumption and calculated the toxicity. Blank tests were carried out to verify the oxygen consumption linear fitting. Toxicity tests were performed using pure/mix organic and inorganic compounds, elutriates from sea- and fresh-water sediment, sewage, dissolved burned compound sub-products. The FM22 test was a good predictor of toxicity for water and soluble samples; the bioassay is easy, low cost and rapid, then usable for routine tests or like a part of a battery of ecotoxicological tests.

FM22 test is a fast, cheap and easy to handle bioassay. Its highlights are:
1- monitoring the O_2 consumption as ΔR (*mitochondrial respiration variation rate*)
2- no ATP production
3- LC50 was calculated.

Bioassay general steps:

- ✓ *beef mitochondria* : prepared and stored at -22 °C (standardized method)
- ✓ *blank test* : to verify the linear fitting of respiration
- ✓ *pure compound toxicity test* : to verify the method sensitivity
- ✓ *internal control for each test* : toxicity is quantified comparing the slope (*m* and *n*) *before* and *after* adding the compound(s)
- ✓ statistical methodology identified a *break-point* in the linear fitting (linear fitting of O_2 consumption was verified by R^2).

METHOD



FM22 end point → ΔR = 1 – *n*/*m*

n = *m* , when testing substance(s) don't show acute toxicity
n < *m* , when testing substance(s) show acute toxic/stressful/disrupting action
n > *m* , when testing substance(s) show a *uncoupling action**, like FCCP behaviour, classifiable as long term toxic action

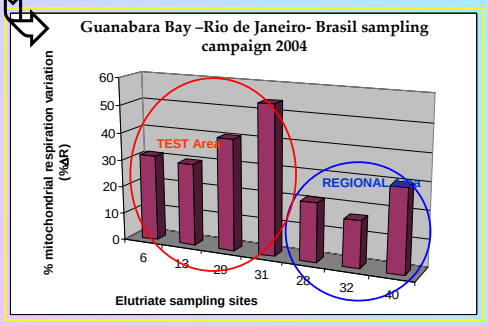
(*) *Uncoupling* means that the flow of electrons across the electron transport chain and the shift of protons from the mitochondrial matrix to the intermembrane space function normally, but the protons to not pass across the F0F1ATPase back to the mitochondrial matrix but directly across the inner membrane; the result is heat production but not of energy in the ATP form.

CASE STUDY

The test was applied in sea water (SW) and in fresh water (FW)

1

To verify acute toxicity of elutriate from polluted sediment (SW)



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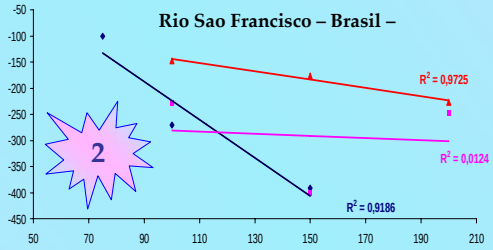

To verify toxicity elutriates, *interface water* (*i.e.* the thin aqueous layer forming after elutriates collection) and *sediment water* (*i.e.* the water present among closed sediment) (FW)

3

To verify toxicity of pure compound solutions

	IC50 (ppb)	Dvs
Zn^{2+}	0,120	0,080
Cr^{6+}	0,085	0,087
Ni^{2+}	0,087	0,056
Cd^{2+}	0,106	0,029
Pb^{2+}	0,099	0,080
Cu^{2+}	0,089	0,106
Benzene	0,397	0,133
DMSO	4515,000	0,153
DDE	13457,000	0,210
Endrin	10545,000	0,183
Dichloromethane	4211,000	0,261
Chlorobenzene	0,516	0,077
1,2-dichlorobenzene	0,476	0,054
1,3-dichlorobenzene	0,499	0,071

- ↳ In the first case study (SW) the acute toxicity can be attributed both to organic inorganic compound present;
- ↳ in the second case study (FW), we exclude the presence of organic compounds, for which the acute toxicity is directly correlated to the heavy metal presence.
- ↳ FM22 is suitable test also to verify to pure compound toxicity (third case).
- ↳ FM22 allows subchronic toxicity study too.

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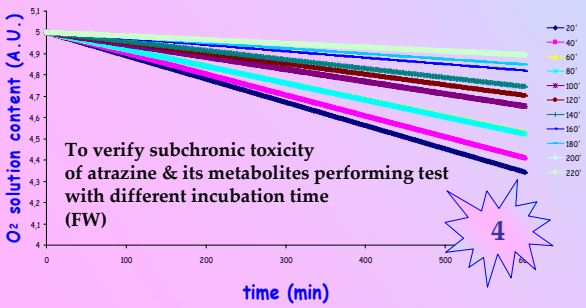

To verify subchronic toxicity of atrazine & its metabolites performing test with different incubation time (FW)

CONCLUSIONS

- The results obtained show that the FM 22 can be considered an excellent tool to be used to verify both acute (normal test) and probable subchronic/chronic toxicity .
- The FM22 test was a good predictor of toxicity for water and soluble samples; the bioassay is easy, low cost and rapid, then usable for routine tests or like a part of a battery of ecotoxicological tests.
- The FM22, therefore, was considered a valid alternative to the test now used, almost as screening test, showing it could be a potential tool for environmental research.

ESSENTIAL REFERENCES

Azzone et al. (1979). *Methods in Enzymology* 55: 46-60.

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Iero et al. (2003) *Chemosphere* 53: 1115-1123.