

# Using the MICREDOX<sup>®</sup> biotoxicity assay to determine contaminant toxicity

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## Aim

The MICREDOX<sup>®</sup> assay has been miniaturised, from a relatively small volume of 17 ml to 200 µl, making the assay more user friendly, inexpensive and able to facilitate a high level of replication. To verify that this miniaturised format will accurately measure the impact of priority contaminants, a group of archetypal toxicants (the organochlorines) were tested using the MICREDOX<sup>®</sup> Direct Toxicity Assessment (DTA) assay.

## Introduction

MICREDOX<sup>®</sup> is a rapid microbial-based assay, originally developed as a rapid Biochemical Oxygen Demand (BOD) assay (Pasco *et al.*, 2001). This assay is characterised by high levels of biocatalyst (microorganisms) and mediator, facilitating a fast reaction in which the microbial oxidation of organic substrate is coupled to the reduction of mediator. The microbially reduced mediator accumulates as a product, registering the amount of bioconversion. Electroanalytical techniques are used to measure the quantity of reduced mediator, giving a direct measure of the microbial respiratory activity that occurred during the incubation (Fig. 1.) (Tizzard *et al.*, 2004).

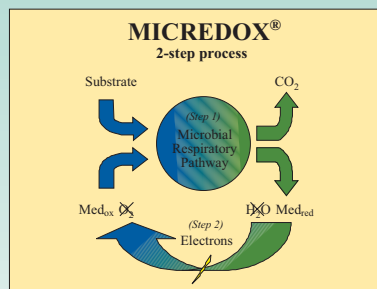


Fig. 1. MICREDOX<sup>®</sup> assay—two step process

MICREDOX<sup>®</sup> has subsequently been adapted to measure the toxic impact of contaminants. In toxicity mode, the electrochemical signal (from the oxidation of microbially reduced mediator) produced by healthy cells is compared to the signal produced by cells that have been subjected to a fixed level of toxicant. The ratio of the electrochemical signal recorded in the presence of toxicant, relative to that recorded in the absence of toxicant, provides an index of the respiration inhibition (Inhibition Quotient, % IQ). EC50 values (i.e. the concentration at which the respiration was decreased by 50%) can be interpreted from a graph of IQs plotted against concentration.

This poster reports the toxicity of mono-, di- and penta- chlorophenols using the miniaturised MICREDOX<sup>®</sup> format with *Escherichia coli* K12 and *Bacillus subtilis* B8.

## Methodology

### Bacterial strain selection

*E. coli* K12 and *B. subtilis* B8 were chosen as these strains had previously been tested in the conventional large scale MICREDOX<sup>®</sup> assay.

### Growth of bacteria

*E. coli* K12 was cultured in minimal salts davis and *B. subtilis* B8 in basal salts buffer, plus trace elements (Herbert salts) and 10 mM succinate. In both cases, cells were harvested after 16 hours of growth at the appropriate temperature, shaking at 200 rpm and with a liquid to air ratio of 1:5.

### Organochlorine selection and preparation

Four chlorophenols commonly used in a variety of industrial processes such as paper-pulp and wool scouring were tested.

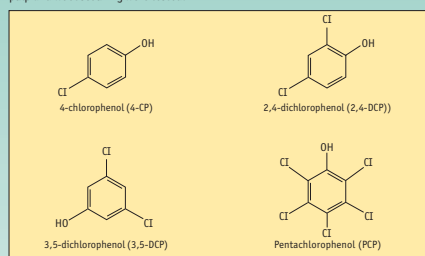
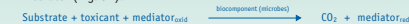


Fig. 2. Chemical structure of the organochlorines studied

Stock solutions (10 x) were prepared in milliQ water and stored in the dark at 4 °C. Before spiking the MICREDOX<sup>®</sup> assay, the chlorophenol solutions were warmed to room temperature.

### MICREDOX<sup>®</sup> Assay

**1. Incubation**  
Microorganisms were incubated with an organic substrate, toxicant and redox mediator (Fig. 3A).



**2. Signal detection**  
The amount of microbially-reduced mediator was quantified by measuring the limiting anodic current (Fig. 3B).

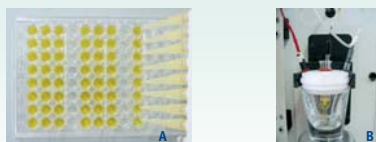


Fig. 3. Incubation and signal detection for MICREDOX<sup>®</sup>  
A) Incubation—assay set up using multi-channel pipette in a 96 well plate with a final assay volume of 200 µl. B) Detection—signal detection is performed by measuring the limiting current at Pt microelectrode, Time Based Amperometry (TBA) analysis.

### Toxicity methods

To determine the EC50, a % IQ value is calculated, as follows:

$$\% \text{ IQ} = \left[ 1 - \frac{\text{Sample} - \text{Endog}}{\text{BOD}_{100} - \text{Endog}} \right] \times 100$$

The % IQ obtained for each individual toxin concentration is plotted against its corresponding concentration. Using a best fit regression line, EC50s can be determined for each toxin.

## Results

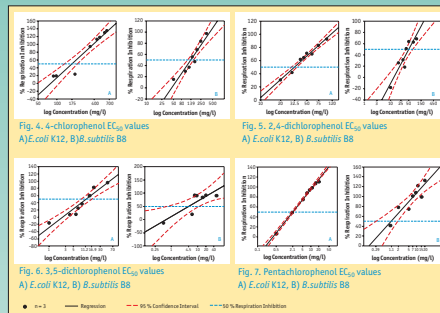


Table 1. EC50 values for chlorophenols using MICREDOX<sup>®</sup> and other commonly used DTA methodology

Method	4-chlorophenol	
	MICREDOX <sup>®</sup> — <i>E. coli</i>	175 (134–220) <sup>*</sup>
MICREDOX <sup>®</sup> — <i>B. subtilis</i>	139 (110–170) <sup>*</sup>	
CellSense— <i>E. coli</i>	200.8	
ToxAlert100 <sup>®</sup>	21.21	
Microtox <sup>™</sup>	42.7	
Method	2,4-dichlorophenol	
	MICREDOX <sup>®</sup> — <i>E. coli</i>	32.5 (29–35) <sup>*</sup>
MICREDOX <sup>®</sup> — <i>B. subtilis</i>	50 (38–70) <sup>*</sup>	
CellSense— <i>E. coli</i>	393	
ToxAlert100 <sup>®</sup>	2.85	
Microtox <sup>™</sup>	101	
Method	3,5-dichlorophenol	
	MICREDOX <sup>®</sup> — <i>E. coli</i>	11.2 (8.2–17.5) <sup>*</sup>
MICREDOX <sup>®</sup> — <i>B. subtilis</i>	4.5 (0.65–11) <sup>*</sup>	
CellSense— <i>E. coli</i>	13	
ToxAlert100 <sup>®</sup>	7.5	
Microtox <sup>™</sup>	4.6	
Method	Pentachlorophenol	
	MICREDOX <sup>®</sup> — <i>E. coli</i>	2.1 (1.8–2.3) <sup>*</sup>
MICREDOX <sup>®</sup> — <i>B. subtilis</i>	1.1 (0.29–2) <sup>*</sup>	
CellSense— <i>E. coli</i>	0.037	
ToxAlert100 <sup>®</sup>	1.07	
Microtox <sup>™</sup>	0.99	

EC50 values reported by: CellSense (Farré *et al.*, 2001); ToxAlert<sup>®</sup> (Farré *et al.*, 2001); Microtox<sup>™</sup> (Ulitzur *et al.*, 2002).

<sup>\*</sup> 95 % Confidence Intervals

## Conclusions

- Miniaturised MICREDOX<sup>®</sup> reports EC50 values similar to those reported by other DTA assays. This miniaturised MICREDOX<sup>®</sup> provides a rapid and reliable DTA of toxicants.
- Confirms previous reports of increasing toxicity with increasing chlorine substitution, with a higher toxicity for meta-substituted chlorophenols compared to ortho-substituted. (Argese *et al.*, 1995).
- *B. subtilis* B8 was, overall, the more susceptible to this group of organochlorines compared to *E. coli* K12.
- MICREDOX<sup>®</sup> is amenable to standard strains of terrestrial organisms for DTA testing.

## Acknowledgement

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The MICREDOX<sup>®</sup> assay is covered by patent PCT/NZ97/00158.

## References

- Argese, E., Bettio, C., Ghelli, A., Todeschini, R. and Miano, P. (1995). *Environmental Toxicology and Chemistry* 14 (3), 363–368.  
 Farré, M., García, M., Trapu, L., Ginebreda, A. and Barceló, D. (2001) *Analytica Chimica Acta* 427, 181–1.  
 Farré, M., Pains, D., Alonso, M., Castillo, M. and Barceló, D. (2001) *Analytica Chimica Acta* 426, 155–165.  
 Tizzard, A., Webber, J., Gooneratne, R., John, R., Hay, J. and Pasco, N. (2004) *Analytica Chimica Acta* 522, 197–205  
 Pasco, N., Hay, J., and Webber, J. (2001) *Biomarkers* 6, 83–89  
 Ulitzur, S., Lahav, T. and Ulitzur, N. (2002) *Environmental Toxicology Journal* 17, 291–296

