

# The effect of pesticide mixtures on *Daphnia magna* gene expression.

Hill, C.J. | Periera, J.P. | Sibly, R.M. | Bolshakov, V. | Gonçalves, F., Heckmann, L-H., Callaghan, A.

## Introduction

Methomyl, a carbamate pesticide (an inhibitor of acetylcholinesterase) and propanil, an anilide herbicide (a photosynthetic electron transport inhibitor) often mix in the environment following crop spraying. *Daphnia magna* acute toxicity tests with a methomyl and propanil mixture revealed strong synergistic effects on immobility. Both synergistic and antagonistic effects on *D. magna* gene expression were detected by cDNA microarray analysis.

## Methods

Neonate *D. magna* <24h old were exposed to methomyl ( $10.5 \mu\text{g l}^{-1}$ ), propanil ( $3.3 \text{ g l}^{-1}$ ), and an equitoxic mixture of the two for 48h (with 4 replicates of each treatment). Total mRNA was extracted, amplified using Amino Allyl Message Amp™ II (Ambion, UK) and labeled with Alexa Fluor™ dyes (Invitrogen, UK). Fluorescently labeled cDNA was hybridised to cDNA microarrays. Each spot on the cDNA microarray is a separate short "expressed sequence tag" (EST) of a *D. magna* gene. Relative expression was normalised to a reference pool (a mix of cDNAs from all treatments) and expression in exposed *Daphnia* was compared to expression in control samples. Spots with >2-fold change in at least one treatment were selected for further analysis (1-way ANOVA  $P < 0.05$  with adjustment for multiple testing). Interactions in the effect of methomyl and propanil on gene expression were revealed through 2-way ANOVAs.

## Results

Of the 15,000 spots on the microarray, 932 showed >2-fold differences between the treatment and control. Of these, 317 spots showed an additive response (Fig. 1, 2); 199 spots showed a synergistic response (Fig. 3, 4); 294 spots showed an antagonistic response (Fig. 5, 6) and 84 spots responded where the effect of methomyl was synergised by propanil or the effect of propanil was antagonised by methomyl (Fig. 7, 8).

## Discussion

Pesticides are often present in the environment in mixtures. Generally, ecotoxicologists study chemical effects individually, yet combined as mixtures, they can be far more toxic. Here we show that a combination of two chemicals applied against two very different pests (insects and weeds) is far more toxic than a simple addition of the effects. The cDNA microarray results reflect the toxic effects (i.e. phenotypic immobility). Most of the spots (representative of genes) that differed from the control responded to both pesticides. Of these, 577 (62%) were directly linked to the phenotypic response. The next step will be to discover *D. magna* gene pathways affected by the propanil / methomyl mixture and to try to understand how the very different modes of action of the two chemicals interact to cause synergy and antagonism in both toxicity and gene expression.

