

Introduction

The data reported forms part of a larger study to investigate the potential effects of pesticides upon headwater stream communities.

Pesticides are developed for their toxicity and specific mode of action on target pest species. It is however well documented that pesticides can affect non-target organisms and some may have an impact upon endocrine-driven functions.

This study specifically considers the potential for oestrogenic effects of pesticides in headwater streams. The work programme was conducted in two main parts that are outlined in Figure 1.

LABORATORY STUDY

LITERATURE SEARCH AND PESTICIDE APPLICATION DATA



IN VITRO OESTROGENIC SCREEN OF PESTICIDES



IN VIVO OESTROGENIC SCREEN OF IN VITRO ACTIVE PESTICIDES

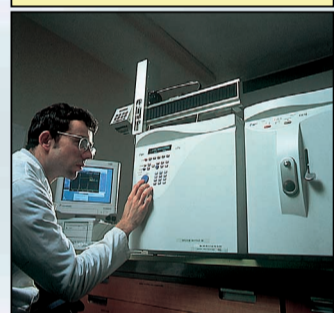


FIELD STUDY

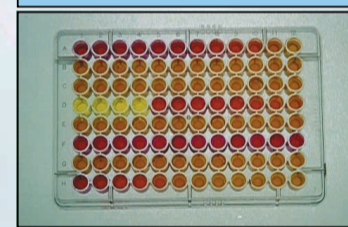
FIELD SAMPLING OF HEADWATER STREAMS



CHEMICAL ANALYSIS OF TARGET PESTICIDES BY GC-MS



IN VITRO OESTROGENIC SCREEN OF FIELD SAMPLES



IDENTIFICATION OF IN VITRO ACTIVE COMPOUNDS BY HPLC AND GC-MS



POTENTIAL OESTROGENIC EFFECTS ASSESSED

In Vitro Yeast Oestrogen Screen

The yeast oestrogen screen is a rapid *in vitro* assay that utilises a genetically modified yeast strain containing the human oestrogen receptor (Ref. 1).

The yeast assay was used to screen a range of pesticides for oestrogenic activity. The results are shown in Table 1.

Table 1 Pesticides screened using the *in vitro* yeast oestrogen assay.

Pesticide name	<i>in vitro</i> Oestrogenic response	<i>in vitro</i> Oestrogenic potency# (A)	Measured environmental pesticide concentrations [ng/l] (B)	Equivalent 17β estradiol environmental concentration [ng/l] (AxB)
Atrazine	No			
Carbendazim	Yes	2x10 ⁻⁸	138 - 1810(1)	3x10 ⁻⁶ - 4x10 ⁻⁵
Carbofuran	No			
Chlorothalonil	No			
Chlorpyrifos	No			
Deltamethrin	No			
Dimethoate	No			
Diuron	No			
Endosulfan	No			
Fenpropimorph	Yes	1x10 ⁻⁴	780(2)	0.08
Isoproturon	No			
Lindane	No			
Paclitaxel	Yes	5x10 ⁻⁵	187 - 609(1)	9x10 ⁻³ - 0.03
Paraquat	No			
Pendimethalin	No			
Permethrin	Yes	3x10 ⁻⁸	5.5 - 55(1)	3x10 ⁻⁷
Propiconazole	Yes	2x10 ⁻⁴	260 - 1240(3)	0.05 - 0.25
Simazine	No			
Tebuconazole	Yes	2x10 ⁻⁴	70 - 170(3)	0.01 - 0.03
Trifluralin	No			

= potency relative to 17β-estradiol.

(1) Environment Agency - National Database of Pesticide Monitoring in the Environment, 1997.

(2) Institute of Hydrology - Rosemaund Transport Study, 1987-1993.

(3) CEFAS and Institute of Hydrology - The Impact of Pesticides on River Ecology, 1999.

The equivalent 17β-estradiol concentrations shown above are two orders of magnitude below the levels shown to produce an *in vivo* oestrogenic response in fish (Ref. 2).

In Vivo Rainbow Trout Oestrogen Screen

An *in vivo* test for oestrogenic activity was also used to confirm that oestrogenic activity measured *in vitro* was not modified.

The level of the yolk protein, vitellogenin (VTG), in male rainbow trout blood plasma 7 days after an intraperitoneal injection of the test chemical was used as a screen for oestrogenic activity (Ref. 3). The results from an initial trial are shown in Figure 2.

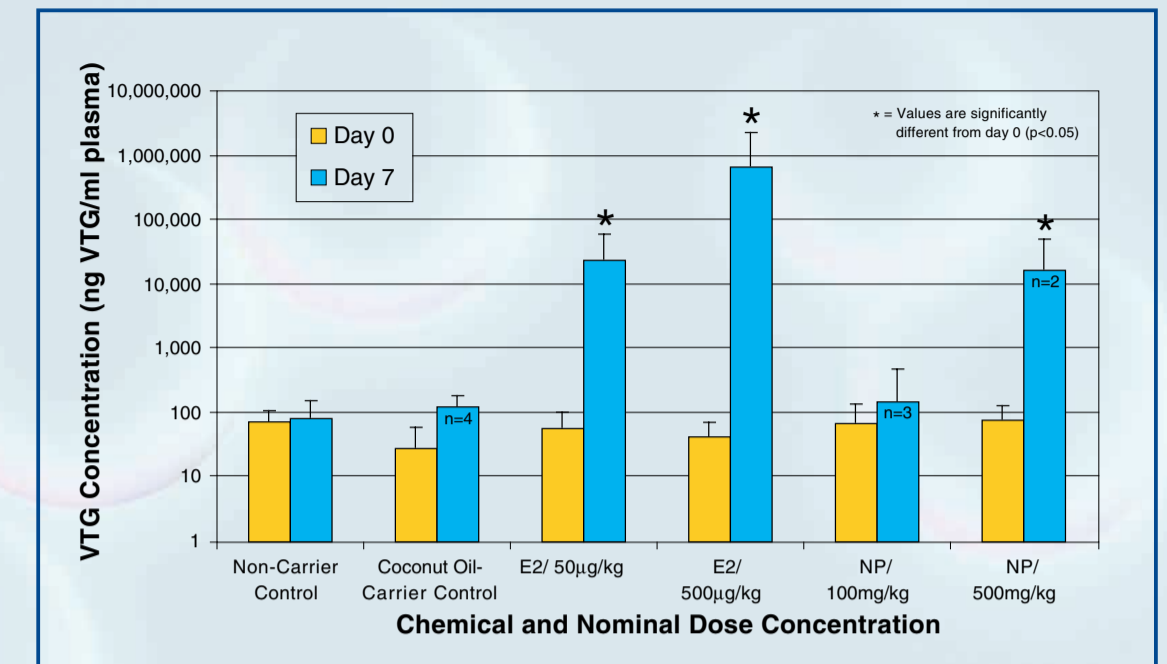


Figure 2. Mean [and 95% confidence limits] VTG induction in male rainbow trout (n=5) after intraperitoneal injection with 17β-estradiol (E2) and 4n-nonylphenol (NP) using coconut oil injection carrier.

Two of the pesticides that showed oestrogenic activity in the yeast assay, propiconazole and fenpropimorph were tested in the *in vivo* assay. Neither compound showed oestrogenic activity *in vivo* at a nominal dose concentration of 250mg/kg.

In Vitro Screen For Oestrogenic Activity In Stormwaters

Many pesticides are translocated to headwater streams draining agricultural catchments. Water samples collected from headwater streams during storm events were therefore tested for oestrogenic activity using the *in vitro* screen. The results are shown below in Table 2.

Table 2 Field samples screened using the *in vitro* yeast oestrogen assay.

Field site name	Sample description	<i>in vitro</i> Oestrogenic response	Equivalent 17β-estradiol environmental concentration [ng/l] ▲
Arable site	Field drain stormwater sample	Yes	6
Arable site	Stream stormwater sample	Yes	0.07
Arable site	Stream non-stormwater sample	No	<0.01
Orchard site	Stream stormwater sample	Yes	0.03

▲ = relative to 17β-estradiol standard, conducted alongside sample extract.

The results in Table 2 show that:

Water samples from headwater streams collected during storm events contain chemicals that produce weak *in vitro* oestrogenic activity.

High oestrogenic activity was measured directly from a field-drain but received considerable dilution upon entering the receiving stream.

Conclusions

A number of pesticides were shown to have *in vitro* oestrogenic activity and many of these have been detected in surface waters.

Water samples collected from headwater streams during storm events have shown oestrogenic activity *in vitro* although none of the pesticides that showed activity in the screening assay were detected in these samples.

Other potential sources of oestrogenic activity in headwater streams include: slurries of animal waste or sewage sludge spread on agricultural land and the breakdown products of nonylphenol ethoxylates used in pesticide formulations (Ref. 4).

References

1. Routledge, E.J. and Sumpter J.P. *Environ. Toxicol. Chem.* 1996, 13, 241-248.
2. Routledge, E.J. et al. *Environ. Sci. Technol.* 1998, 32, 1559-1565.
3. Christian, L.B. et al. *Marine Environ. Research.* 1998, 46, 137-140.
4. Thomas, K.V. et al. *Water Research.* IN PRESS.