The Impact of Pesticides on River Ecology
Phase II, A Study of Headwater Streams

Technical Report
P423

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R&D Technical Report P423

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EXECUTIVE SUMMARY

Background

1. Previous pesticide transport studies have demonstrated that organisms in headwater streams may be exposed to transient but high concentrations of pesticides following storm events. The communities in headwater streams (defined here as stream length within 2.5km of the source) may therefore be at risk from the effects of pesticides following storm events. Estimates suggest that headwaters may represent 62% of freshwater habitat in the UK and 20% of species may be exclusive to this habitat. It was therefore considered important to make an assessment of the potential impact of pesticides upon headwater streams communities.
2. The key objectives of the project were: (i)To identify headwater streams draining catchments most likely to be at risk from pesticide runoff.
   (ii) To monitor background and peak concentrations of pesticides known to be in use in the catchments. (iii) To develop appropriate sensitive bioassay procedures for deployment at selected field sites and to associate the measured pesticide concentrations in streams with effects in bioassays, and establish any links with effects observed in indigenous organisms.
3. Four headwater streams draining cracking clay catchments with different agricultural usage were studied over a three-year period. Most fields were drained and transport of pesticides to streams was expected to be close to maximal. Two were arable and one consisted of orchards and hop gardens. A small stream draining the fourth site, on which there was a high grazing intensity of sheep, was monitored for the potential effects of sheep-dip pesticides that were disposed of to adjacent land.
4. The main focus of the study was the potential for the biological effects of pesticides upon the indigenous flora and fauna present in headwater streams, following storm events. Automatic sampling units triggered by increased stream flow were used to sample transitory increases in flow following storm events.
5. Pesticides which are known to be most readily translocated in drainage water and which were applied in a catchment prior to a storm event, were monitored for in automatic water samples.

Methods

6. Bioassay procedures were developed to determine the presence of biologically active compounds during storm events when pesticide concentrations were expected to be at their peak. Some of the procedures involved direct toxicity tests of the water sampled during storm events, comparing survival or growth of standard test species. In addition, the survival and feeding rate of test species that were caged at each site were also measured in order to provide a more realistic assessment of exposure to compounds that might be present in storm waters.
7. Organic compounds present in stream water samples taken during storm events were concentrated on solid-phase columns. The concentration factor required to produce biological effects was determined in standard toxicity tests of diluted solvent extracts of these columns (SPEs).
8. Some of the stream samples produced acute toxicity without pre-concentration. The SPE technique was however useful in highlighting those samples that were most likely to produce chronic toxicity, particularly when no effects could be detected in tests of neat samples.

9. Changes in the numbers and species of invertebrates sampled in drift nets at each site were monitored weekly and each month the invertebrates living on the streambed were also sampled.

Results

10. A total of 71 storm events were sampled for the four sites over three years. Twenty-six compounds were analysed for across all four sites but only 12 pesticides were detected in water samples collected during storm events. Nine of these compounds were herbicides and the remaining three fungicides.
11. Based on the measured concentrations of pesticides the potential for effects on plants in a storm water sample was more accurately predicted than effects on animals. This probably reflects the fact that the algae are particularly sensitive to herbicides that were relatively well detected using the extraction and analysis techniques applied to the samples. Low concentrations of the more persistent metabolites of insecticides such as endosulphan sulphate and other compounds such as nonylphenol were not routinely looked for in samples but were found to make a major contribution to toxicity and are in general more toxic to animals than plants.
12. The predicted toxicity for samples indicated that four out of 19 for one of the arable sites and eight of 21 for the hop garden and orchard site fell within a factor of ten to 100 of producing acute toxicity to the freshwater crustacean *Daphnia magna*. For the green alga *Raphidocellis subcapitatum*, five of 14 events for one arable site and three of 17 events for the hop garden site fell within a concentration factor of one to ten of producing acute toxicity.
13. On two occasions acute toxic effects were measured in bioassays deployed in situ in a stream at one of the arable sites and also in a field drain sample at the same site on another occasion. These events are believed to have occurred as a result of the drift of insecticides during application in a field adjacent to the monitoring site.
14. Use of multi-variate statistical analysis indicated that there were differences in the invertebrate community present at each of the main study sites relative to field controls. At one site, studied in more detail, a gradient of impact upon the invertebrate community was apparent. The observed reductions in some species such as the crustacean *Gammarus pulex* appeared to be quite localised and were potentially associated with adjacent hop-gardens and orchards.
15. In the stream draining the catchment on which there was a high intensity of sheep grazing none of the pesticides used in dip formulations that were analysed for were detected. It was confirmed that some landowners disposed of spent dip in the catchment however no biological effects were observed at this site during the monitoring period and biological quality of the stream remained good throughout the monitoring period. It must be noted however that the first major rainfall events sampled were during November and the latest period during which sheep would have been dipped and spent dip disposed of would be July-August. It is therefore likely that pesticides disposed of in dip would have degraded to a large extent in the soil before they could be translocated to the stream during a storm event.

Conclusions

16. A number of valuable biological monitoring techniques were developed. The combination of solid-phase extraction of storm event samples with subsequent testing using bioassay procedures provides a powerful approach for the identification of potential biological effects of pesticides upon stream communities. Multivariate analysis of invertebrate sample data allows more of the information collected using traditional survey methods to be included in an assessment of the impact of pesticides upon the stream fauna. Both approaches are recommended in future monitoring programmes.
17. In summary, some pesticides generally occur at sufficient concentration and frequency in headwater streams that drain agricultural catchments to produce chronic biological effects. This effect of contaminated drainflow with the occasional occurrence of higher pesticide contamination levels in streams following spray drift has almost certainly led to degradation in the biological communities present in the streams studied.

1. INTRODUCTION

1.1 Background

The Rosemaund pesticide transport project (Hack 1995, Matthiessen *et al*. 1994, and Turnbull *et al*.1997.) demonstrated that water draining from a primarily agricultural catchment can contain sufficiently high concentrations of pesticides to result in the mortality of aquatic organisms. Headwater streams, defined as the first 2.5km of a watercourse (Furse, 1995) are of primary interest since the organisms they contain are likely to be exposed to the highest concentrations of pesticides from adjacent land, before significant dilution or degradation occurs. Organisms inhabiting headwater streams may be able to survive transiently high concentrations of pesticides, but there is little information available to allow the longer-term population effects of such exposures to be predicted.

Headwater streams represent a significantly large percentage of the habitat available to freshwater aquatic organisms (estimated 62% by length, Furse *et al*. 1994) and as such are an important natural resource which requires careful management and protection. Development of a successful approach towards the management of this resource requires the collection of information which will enable the likely degree of pesticide exposure and its biological effects, at both the individual and community level, to be measured.

1.2 Objectives

The primary objective of this project is to develop and deploy methods to determine whether pesticide use has an impact on the aquatic species and communities present in headwater streams. The four specific component objectives identified to achieve this primary objective are :

- To identify headwater streams draining agricultural catchments on which the surface geology is cracking clay soil and drainage water moves largely via field drains to the stream. Cracking-clay soil is prone to bypass flow, this can result in high concentrations of pesticide being rapidly translocated to headwater streams in this type of catchment. Similar soils with underdrainage form 45% of agricultural land in the UK (Cannel *et al*., 1978) and 32% are dominated by bypass flow (Boorman *et al*., 1995)

- To monitor background and peak concentrations of pesticides known to be in use in the catchment.

- To develop appropriate sensitive bioassay procedures for deployment at selected field sites.

- To associate the measured pesticide concentrations in streams with effects in bioassays, and establish any links with effects observed in indigenous organisms.

2. RESEARCH STRATEGY

2.1 Experimental Design

The primary objective of this study was to determine whether pesticide use has an impact upon headwater stream communities. A subsidiary objective was to develop a range of bioassays that could be deployed as monitoring tools. Suitable field sites were identified and water-sampling equipment was deployed to take samples from headwater streams during periods of high rainfall and increased flow. Samples of indigenous invertebrates were collected at intervals during the study and various bioassays were either deployed in situ or used in the laboratory to test water samples collected from the streams or from field-drains.

2.1.1 Selection of headwater streams

Streams potentially impacted by pesticides were initially identified by the Environment Agency, and landowners prepared to co-operate with the study programme were identified by the National Farmers Union (NFU). The initial intention was to include two arable sites, one catchment on which hops and/or orchard fruits are cultivated and one site on which sheep-dipping and dip-disposal occurs. At the recommendation of the funding agencies a source of horticultural effluent was not considered further. The selection of sites was based on the percentage representation of these land use types in the U.K., on the potential environmental impact of the pesticide combinations used in each form of agricultural practice, and on the presence of heavy cracking clay with field drains.

After initial acceptance of a site based on the above criteria, site visits were conducted so that ease of access and siting of sampling equipment could be established. At the same time, sites were evaluated for confounding influences upon stream quality, such as septic tank discharges, discharges from the storm overflows of small sewage treatment works, or runoff from urban areas, roads and railways. Approximately 40 sites were considered; these were narrowed down to two suitable arable sites and a site on which both apples and hops are grown. The ‘sheep-dip’ site proved more problematic. An early site established on the River Tillingham in East Sussex proved unsuitable since the channelised nature of the river and the use of sluice gates made it impractical to deploy bioassays and sampling equipment effectively. Other sites on which tenant farmers were co-operative were not made available due to objections of land agents or owners. To resolve the difficulty of identifying a suitable site it was decided to collect samples from the River Tillingham during increased flow, for analysis of the main compounds used in sheep-dipping. The intensity of sheep rearing in the catchment was also calculated to enable an estimate of potential inputs of dipping chemicals to the River Tillingham. The sampling point on the River Tillingham was designated site B.

The two arable sites selected were both situated in Essex, these were the Fridaywood and Rye Farms near Colchester, collectively site A (Figure 2.1) and Dollymans Farm near Wickford, site D (Figure 2.1). Site C (Figure 2.1) Harpers Farm near Tunbridge grows both hops and apples within the catchment. Curtisden stream drains the catchment and subsequently joins the Lesser Teise river.

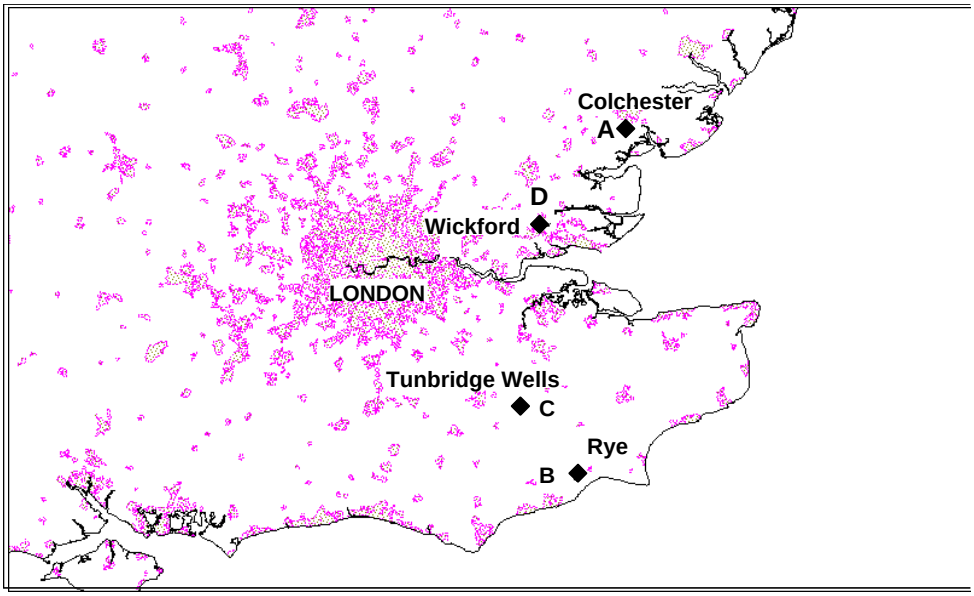

Figure 2.1. Map showing general position of each of the field sites selected Sites A-D.

Site A is situated 5.5 kilometres from the town of Colchester (catchment NGR 970190 - 990200, Ordnance Survey Landranger 168, 1:50000). The study stream, a tributary of Romans Brook, is immediately bordered by fields owned by Rye and Fridaywood Farms (approx. area 12 km^2). The stream ranges between 0.25-0.75 m width and depth of five to ten centimetres at base-flows and the sampling point is two kilometres from the source. The main crops during the 1997 season were field beans wheat and oilseed rape. Generally the soil texture is silty clay derived from the flood plains of the River Blackwater. The fields are under-drained using pipes set at a depth of 1m.

The associated control stream Birch Brook, is situated approximately two kilometres from the main site and is of a similar size (catchment NGR 000220 - 020210 Ordnance Survey Landranger 168, 1:50000), the land immediately adjacent to it primarily grassland with small areas of hawthorn and gorse, being used as a military training area and therefore experiencing little potential for pesticide contamination in drainage water.

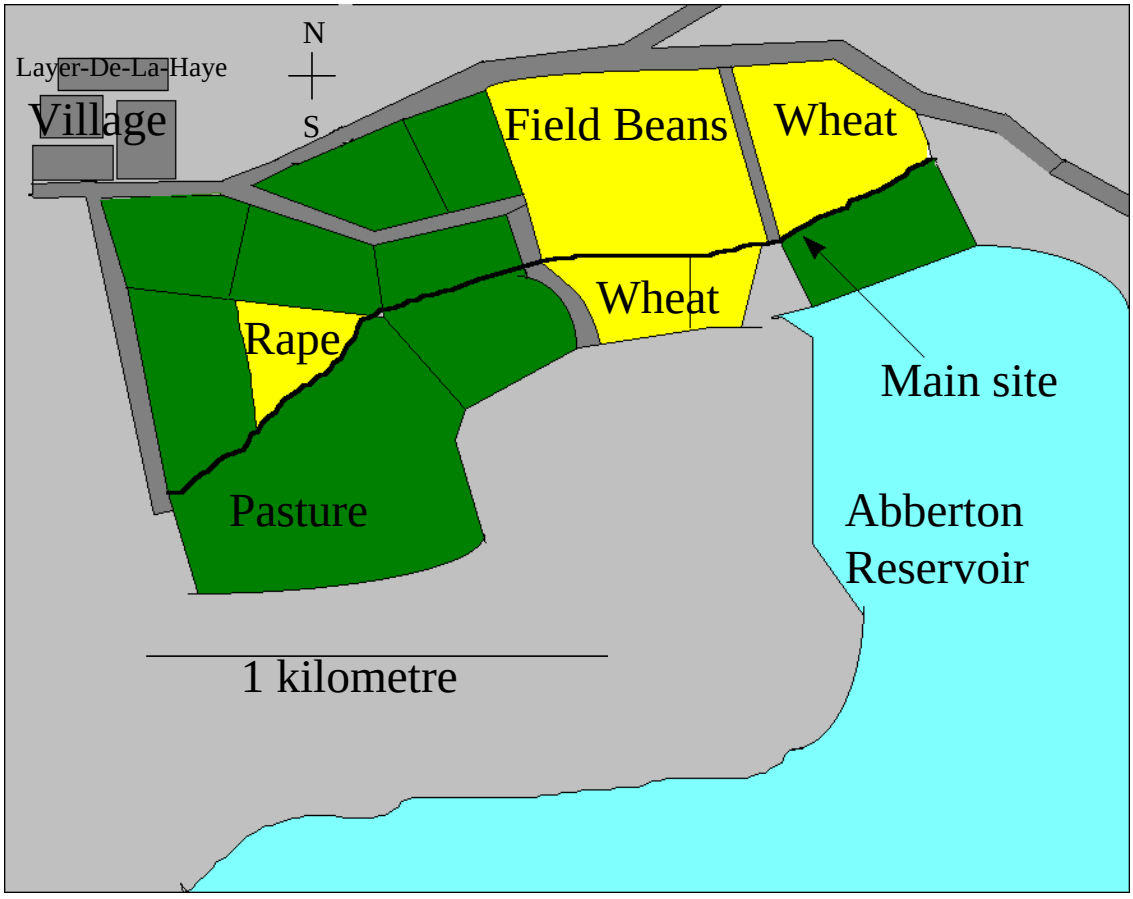

Figure 2.2 : Map showing position of stream site and surrounding crop distribution, Spring / summer 1997 at Rye Farm near Colchester in Essex (Site A).

A range of crops were grown at Rye Farm (Site A) between spring 1997 and spring 1999. The crops grown at the site are shown in Table 2.1.

Table 2.1 Crop types grown at Site A arable, between spring 1997 and spring 1999. The field numbers refer to those indicated on Figure 2.2

Field	1997 Season	1998 Season	Spring 1999
1	Rough pasture	wheat	wheat
2	oilseed Rape	linseed /sugar beet	oilseed rape
3	field Beans	wheat	wheat
4	wheat	wheat	wheat
5	wheat	wheat/rape(September)	oilseed rape
6	wheat	wheat	wheat

Site C is situated 15 kilometres from the town of Tunbridge Wells (NGR 726406, TQ64/74). The Curtisden Green stream drains the immediate catchment (approx. area 14 km^2) on which Harpers Farm is situated. There are several other farms on the catchment owned by the owners of Harpers Farm and two other landowners. Curtisden Green stream is 0.5 - 2 metres wide with a baseflow depth range between 5 - 30 centimetres and the sampling point is 2.5 kilometres from its source. The stream bed is clayey silt with overlying shingle. The stream is fed from several Chalybeate (iron-rich) springs. The main crops during 1997 were hops and apples although there is an area used for soft-fruits at the point just before the stream joins the Lesser Teise. The control stream is situated three kilometres away from the main site near the village of Cranbrook (Catchment NGR 720410 - 740390, Ordnance Survey Pathfinder series 1229 and 1249 respectively). It is similar in form to that on the main site, there is some agricultural usage on the surrounding land (Fodder crops and grazing) but potentially only a small amount of drainage from this will reach the control site because of the direction of slope. Like the main site, the control stream is spring-fed from Chalybeate springs.

A wide range of pesticides was applied to the orchards and hopgardens at the Harpers Farm (Site C) between spring 1997 and spring 1999. The main applications are shown in the result section. The fields marked with an asterisk are those orchards and hopgardens to which the application data relates. This essentially includes all those that lie immediately alongside the Curtisden Green stream. During 1998 several of the fields used for growing hops were grubbed up. The general position of these is indicated on Figure 2.3.

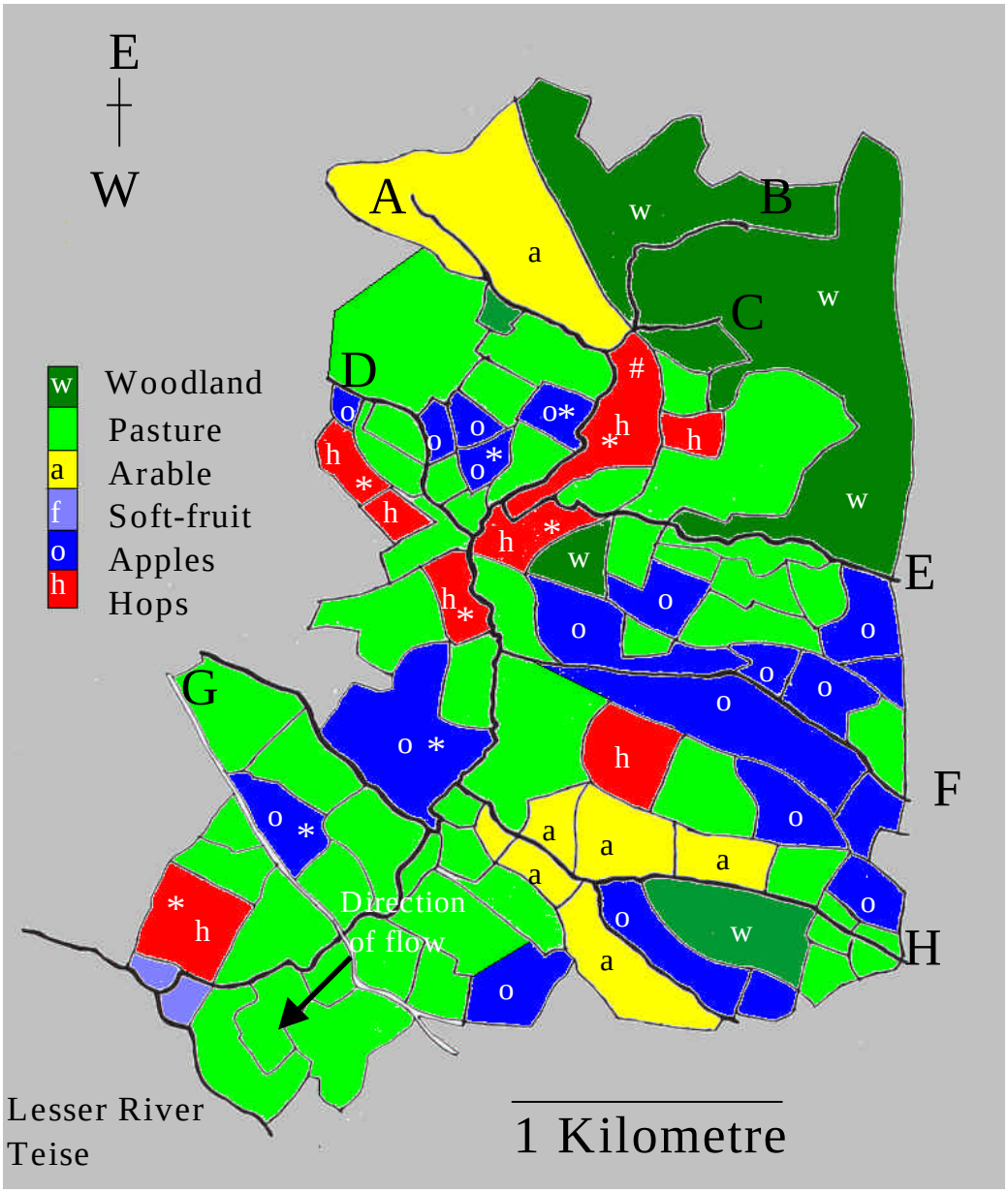

Figure 2.3 Map showing position of stream sites and surrounding crop distribution, spring / autumn 1997 for Harpers Farm. (A - H feeder tributaries of Curtisden Green Stream) * fields to which the application data relates. # the end of this field was grubbed up during 1998.

Site D is situated three kilometres from the town of Wickford (catchment NGR 760910 - 770920, Ordnance Survey Pathfinder 1142). Two unnamed streams drain the Dollymans Farm site (approx area eight km^2). The stream at Doublegate Lane was originally selected as the main site with the Fanton Hall stream as the associated control. This comparison was valid for the 96/97 season but rotation of crops on the site meant that the control stream has several adjacent fields used for cereal crops in the 97/98 season. The stream at Doublegate Lane is 0.5 – 1 metres width with a baseflow depth range between 5 - 15 centimetres and the sampling point is three kilometres from its source. The stream-bed is clay with overlying shingle. The control Fanton Hall stream is similar in form to that on the main site. The soil is a heavy clay loam that was under drained at a depth of 0.8 metres (≈ 40 m spacing). The main crops during 1997 to 1999 were maize, linseed and wheat. Pesticide applications at this site for spring 1997 to spring 1999 are shown in Table 2.3.

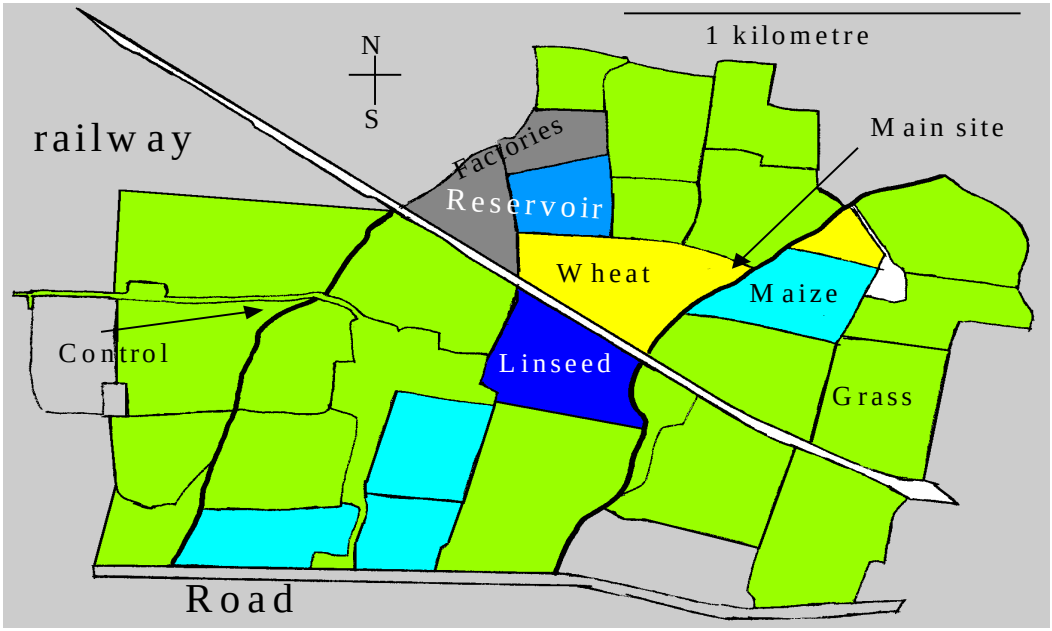

Figure 2.4 Map showing position of stream sites and surrounding crop distribution for the Dollymans Farm (site D)

An outline of the analytical methodology used and detection limits for the pesticides analysed are shown in Table 2.2.

Table 2.2 Analytical methods used in the analysis of water samples for a selected range of pesticides known to be applied in 1997 to 1999 at each of the sites

CHEMICAL	DETECTION LIMIT (µg/l)	EXTRACTION	QUANTATATIVE METHOD
atrazine	0.05	SPE C18 Cartridge	HPLC, UV Detector (acetonitrile/H_20)
chlortoluron	0.07-0.13		
cyfluthrin	0.13		
cypermethrin	0.13		
diuron	0.04-0.19		
isoproturon	0.08-0.13		
simazine	0.06		
2-4-D	0.14-0.28	H_2SO_4/dichloromethane/ SPE Oasis Cartridge	GC-MS SIM (ethyl acetate)
fluroxypyr	0.05-0.1		
MCPA	0.06-0.14		
MCPB	0.06-0.14		
mecoprop	0.14		
atrazine	0.1	Dichloromethane/ SPE Oasis Cartridge	GC-MS SIM (ethyl acetate)
chlorfenvinphos	0.1-0.3		
chlopyrifos	0.04-0.6		
diazinon	0.05-0.3		
fenpropimorph	0.02-0.14		
lindane	0.03-0.08		
metalaxyl	0.1-1.2		
penconazole	0.2-1.2		
pirimicarb	0.03-0.2		
propetamphos	0.05-0.3		
simazine	0.1		
trifluralin	0.1-0.3		
bupirimate	0.05-0.13	SPE C18 Cartridge	GC-MS SIM (ethyl acetate)
diflufenican	0.05-0.1		
propiconazole	0.5-1.2		
pyrasaphos	0.2-0.6		
tebuconazole	0.02		

A control site for the ‘sheep-dip’ catchment (site B) near Rye in Sussex was identified at the beginning of the study period and samples of stream invertebrates were collected to provide a baseline for comparison of community structure with that in the River Tillingham. Chemical analysis of water samples for this site focused on the organophosphorus insecticides diazinon and propetamphos and the pyrethroid insecticide cypermethrin.

2.1.2 Sampling programme

At each of the main sites an automatic data logging system was used to record dissolved oxygen and temperature in each stream. Rainfall was also recorded using an automatic gauge. A stream height monitor operating on a pressure switch was used to measure stream height. Water samplers were triggered to sample from the middle of the stream and at mid-water depth, at a pre-set increase in stream height (corresponding to a rainfall event of approximately five millimetres over a four hour period). Each sampler collected 24 one-litre samples into solvent-rinsed brown glass bottles, and these samples are used for analytical determinations. A duplicate set of water samples was also collected at each of the main sites for bioassay. The main samplers at each site were set to collect samples between September of one year and the middle of May in the following year. These sampling periods reflect the time of year when the highest seasonal rainfall generally occurs.

The sampling programme commenced in spring 1997 and was completed in spring 1999. Samples for pesticide analysis were collected on 54 separate occasions when automatic sampling equipment was triggered during increased stream flow. The analytical methods used in this study enabled the samples to be screened for the presence of 26 active substances.

2.2 Biological techniques employed in the study

2.2.1 General approach

A range of biological techniques were employed in this study in order to increase the likelihood of detecting potential effects of pesticides present in the headwater streams studied. Measuring the effects of transient high concentrations of pesticides which occur following storm events was the main objective of the project therefore several approaches were taken to enable appropriate samples to be collected for testing:

(i) Automatic water samplers were used to collect 24 samples at hourly intervals during increased stream flow following heavy rainfall. In some of the bioassay procedures organisms were directly exposed to water sampled automatically during storm events. This approach was also adopted for samples collected between storm events to determine whether toxicity due to other types of input was present in the headwater streams. Field drains were also sampled during periods when increased flow from the drains made sampling practical and samples were directly tested in bioassay procedures.

(ii) Many pesticides that have been detected in field drainage are present at concentrations that do not produce acute toxic effects. In order to determine how far below the acute toxicity threshold the trace concentrations of pesticide mixtures in field drainage were, bulk water samples of 16-20 litre (from the automatic sampler, field drains and occasional samples between events) from each site were passed through solid-phase extraction columns (SPE). These columns extract (or trap) organic compounds of a large range of polarities. The trapped or extracted compounds on the column can be re-mobilised by passing an appropriate solvent through the column. The re-mobilised or extracted samples in the solvent carrier can then be reconstituted in a small amount of relevant bioassay test medium. This procedure effectively concentrates toxicity and therefore enables the

presence of low levels of toxic compounds (which may produce chronic toxic effects with prolonged exposure) to be discriminated by acute toxicity test procedures. A range of bioassays and screens were therefore applied to the sample at the increased concentration factor to allow for greater sensitivity in the detection of toxic organic compounds.

(iii) In order to integrate the effects of transient high concentrations of toxic compounds which may occur during storm events with the natural rigors of temperature change, increased flow etc. some of the bioassay procedures involved *in situ* deployment of the tests in the headwater stream.

(iv) Pesticides have different affinities for adsorption to soil particles and this may vary dependent upon the nature of the particles and the soil environment. Factors such as soil pH for example have an important influence on the mobility of compounds. Compounds which are strongly bound to soil particles, particularly those in association with colloidal materials, may however be transported in field drainage and ultimately become associated with headwater stream sediments. The bioavailability of these particle-associated compounds in stream sediments depends on the nature of the particle and whether an organism burrows in the sediment or lives on the sediment surface and whether it ingests sediment in its mode of nutrition. Due to the fact that some of the pesticides applied to the study sites are known to become strongly adsorbed to soil particles (e.g. cypermethrin) stream sediment samples were also collected on some occasions for testing in the laboratory. In these procedures the test organism was allowed to establish and grow in the sediment. Impoverished growth, mortality or various biochemical changes were used as endpoint measures to indicate the presence of biologically significant concentrations of contaminants in the sediment.

(v) The main objective of the study was to determine if pesticides present in headwater streams following storm events effect the structure or functioning of the headwater stream community. Indigenous macroinvertebrates were, therefore, also sampled at intervals during the study in order to look for relationships between changes in numbers or diversity and the occurrence of storm events containing measurable concentrations of pesticides.

(vi) Some preliminary work was also conducted to determine the viability of using periphytic algae (those attached to surfaces in the stream) to detect the effects of transient concentrations of pesticides.

Table 2.6 at the end of the section describing methodology summarises the bioassay techniques used.

2.2.2 Bioassays applied directly to water samples

In addition to chemical analysis for specific compounds, 16-20 litre water samples were collected automatically from the stream draining each site during runoff events or on some occasions directly from field drains. For some of the bioassay procedures samples were bulked before testing on other occasions samples collected at hourly intervals were tested separately.

Raw water samples (not solvent extracted or pre-concentrated on solid-phase columns) were initially screened for toxicity using a modification of the *Daphnia magna* OECD acute test guideline 202 (OECD guideline,1984) which involved the use of a small volume testing system (five animals in five mL of test solution). *Daphnia magna* are exposed to water samples collected from each site during a storm event. The percentage mortality of animals exposed to water samples was noted after 48 hours and compared between sites and controls.

Raw water samples were also tested for the presence of acetylcholinesterase inhibiting compounds using the freshwater amphipod crustacean *Gammarus pulex*. Acetylcholinesterase (AChE) is involved in the regulation of nerve impulses and is known to be inhibited by specific groups of insecticides. The acetylcholinesterase assay has been implemented to provide a sub-lethal bioindicator to detect the presence and bioavailability of organophosphorus (OP) and carbamate pesticides at low concentrations. These pesticides inhibit the action of AChE, eventually causing mortality. Some of these compounds could potentially occur in water draining from some of the catchments being studied. The AChE assay was deployed in two phases, with both a field and a laboratory study being conducted.

Test organisms *Gammarus pulex*, were collected from the River Ter in Essex (considered a clean source). Animals retained on a two-millimetre sieve were used for the assay. In the laboratory, animals were maintained in reconstituted freshwater at a constant temperature of 15±1°C and fed on conditioned maple leaves.

The AChE assay used in this study was based on a colourimetric method originally described in by Ellman and Featherstone (1961). This method has been adapted for use on a microplate reader. The AChE enzyme breaks down a substrate compound Acetylthiocholine Iodide (ACTC). Substrate concentration can be measured by the absorbance of the reaction mixture, therefore reduction in the concentration of substrate can be related back to the amount of AChE activity in the sample (based on rate of substrate breakdown). Since the amount of AChE activity is related to protein content of tissues a protein assay is also conducted in order to normalise the AChE activity values across samples.

To perform the assays, ten *G. pulex* head capsules are homogenised, on ice, in 500µl pH7 buffer with Triton X100 (a non-ionic surfactant used to dissolve membrane-bound AChE). The crude homogenate is then centrifuged at 10,000 rpm for 20 min at 4°C. This supernatant is then used to determine AChE activity and protein content. The supernatant is kept on ice until assayed or can be stored at -80°C until used. The AChE assay is performed with all the reagents at 25±1°C. The microplate is read at a wavelength of 412 nm and optical density (OD) readings taken every 15 seconds for one minute to produce a OD min^{-1} value.

The protein content of the supernatant is determined by a dye-binding technique using a colorimetric reaction that is proportional to protein concentration. The AChE activity can now be calculated giving final units of nmoles (ACTC) substrate hydrolysed/ min/ mg of protein. In this way AChE activity in animals exposed at field sites may be compared with unexposed G. pulex maintained in laboratory culture.

Site C (Curtisden Green Stream) was initially chosen for AChE monitoring as the use of chlorpyrifos, an OP pesticide, had been confirmed in that catchment.

Storm event samples were tested using the *Gammarus* AChE assay. Twenty-four hourly samples from the automatic water samples (taken during increases in stream flow) were bulked into four six hour samples. Batches of thirty animals were exposed to these samples for 24 hours after which three groups of ten head capsules were sampled and processed as previously described. The results of the field and laboratory studies are described in the result section.

In addition to animal based bioassays, samples were also screened using the green unialgal species *Raphidocellis subcapitata* in a modified version of the ASTM standard test (ASTM, 1987). The main difference in the procedure was the use of a micro-well test system (each test well was 300ul in volume) and a higher initial algal inoculation density. This assay measured the growth potential of the green alga *Raphidocellis subcapitata* using cell density (cells/ml). A standardised initial density of 50,000 cells/ml was used and the end point of the assay was the increase in cell density after 96 hours incubation at 24°C ± 2° with constant illumination. All cell densities were determined using a Coulter type electronic particle counter.

Twenty-four hourly automatic water samples taken during increases in stream flow were bulked into four six-hour samples. From these, 100 ml was extracted and filtered through a 0.45µm membrane filter to remove the natural algal population. Two replicate 30ml samples were then taken and spiked with nutrients, in order to mask the background effect of natural levels of nutrients on growth. The 0.5ml spike gave final concentrations in the assay of 1.0 mg/l nitrogen and 0.05 mg/l phosphorus. *Raphidocellis subcapitata* was prepared by centrifuging 100ml of culture medium at 1000g for five minutes, rinsing with artificial soft water and re-centrifuging. The pellet of cells was again re-suspended in artificial soft water (25ml), and cell density determined so that an appropriate inoculum could be added to the 30.5 ml samples to produce a starting density of 50,000 cells/ml. Internal controls consisted of an un-spiked composite of samples from 1-24 hours and a positive control of algal growth medium (MBL) to confirm the viability of the algal culture and the adequacy of test conditions.

After incubation, the cell numbers in each sample were counted on an electronic particle counter, and the cell numbers in test and control samples were compared.

2.2.3 Bioassays of solid-phase extracts

Bulk samples of water from the automatic samplers, field drains or samples between storm events were passed through solid-phase columns to extract toxic organic compounds which could then be re-extracted and tested at higher concentration factors.

Basically, 20L samples were passed through a C18 (5g; IST) and ENV+ (1g; IST) SPE columns in series. Both columns had been previously solvated by the addition of methanol (10mls) and water (10mls) in order to activate the extraction media. The columns were then stored at –20°C.

Before extraction of the columns they were allowed to thaw at room temperature. The mid-polar to polar organics were recovered by passing methanol (5ml) through the extraction medium that was allowed to soak for ten minutes. This was then repeated. The non-polar organics were recovered by repeating the process with dichloromethane (DCM; 2 X 5mls).

All fractions were then combined and reduced in volume to 500µl (N_2 @ 40°C; Turbovap). This extract was reconstituted in 10mls of distilled water. The extract was divided up so that a range of bioassays could be conducted.

SPE extracts were screened for toxicity using four main bioassay techniques:
(i) The *Daphnia magna* acute toxicity test
(ii) A growth inhibition test using the green unialgal species *Raphidocellis subcapitata*.
(iii) A 48 hour bioassay procedure using the small aquatic macrophyte *Lemna minor*.
(iv) An acetylcholinesterase (AChE) inhibition assay using the midge larvae *Chironomus riparius*

Daphnia Immobilisation Bioassay
For this procedure three millilitres of extract was taken and made up to 60mls in Elendt M7 medium. This gave a final concentration factor of 100. The bioassay was performed following the main principles of the OECD acute test guideline 202 for *Daphnia magna*. The main exception was the use of a reduced test volume, 20 mls per treatment level. The reduced test volume enabled the use of higher concentration factors that effectively increased the detection limits for biological effects.

Algal Growth Inhibition Bioassay
For the algal assay three millilitres of extract was taken and made up to 60 mls in MBL medium. This also gave a final concentration factor of 100. In addition to a reduced test volume a larger algal inoculum (50000 cells per ml) was added to each dilution of the test solution and the test was run from 48 to 72 hours. The test conditions were otherwise maintained according to the ASTM standard test guideline.

Lemna Conductivity Bioassay
Due to the different modes of action of various herbicides it is important to use aquatic macrophytes as well as algal test species when setting up bioassay procedures. For this reason a method using *Lemna minor* was also developed for testing SPE extracts. For the assay using *Lemna*, two millilitres of extract was taken and made up to 40mls in distilled water. This gave a final concentration factor of 100.

The sub-lethal effects of photosynthesis-inhibiting herbicides have been previously studied by measuring oxygen evolution or photosynthetic electron transport in isolated chloroplasts or plant cell cultures. Such techniques require specialist skills, expensive equipment and may not be relevant to effects on whole plants. One method that avoids these problems is to use the ability of this class of herbicides to antagonise the activity of dipyridinium herbicides such as paraquat (Parker 1965, Yanase *et al*. 1990). Paraquat requires a supply of high-energy electrons from photosynthesis in order to produce the hydroxyl radicals that are the mechanism of its herbicidal activity. These destructive radicals cause cell lysis, which is easily measurable in aquatic plants as an increase in the conductivity of the surrounding water. Any inhibition of photosynthesis will decrease the electron supply and thus decrease the rise in conductivity relative to a paraquat-only control.

The method of Yanase *et al*. (1990) for cucumber leaves was adapted to the use of *Lemna* in a series of feasibility tests. In order to obtain a sufficient rise in conductivity it was found necessary to use 90mm diameter crystallising dishes containing 30ml of water, the surface of

which was covered in *Lemna* minor. This gave a constant area, as opposed to number, of plants. Ten micromols of paraquat was the final concentration in each of the assay vessels, giving a quick (48 hr) and large increase in conductivity. The assay was run in an incubator at 20°C and a light intensity of 4300 lux. Conductivity readings for the test solutions were made at daily intervals. A syringe was used to extract of c.7.5 ml of the water from underneath the floating plants this extract was placed in a beaker that was just larger than the conductivity probe. Once a conductivity reading was taken the water was returned to the assay dish. All dishes were covered in transparent film to minimise evaporation.

In the assay procedure *Lemna* fronds were exposed to a fixed concentration of the herbicide paraquat in addition to one of a range of dilutions of an SPE extract. The paraquat causes cell lysis of the *Lemna* fronds, increasing the electrical conductivity of the test medium. If photosynthesis-inhibiting herbicides are present in the SPE extract these counteract the effect of the paraquat and result in a smaller rise in electrical conductivity in the test medium. A test procedure using smaller numbers of *Lemna* fronds and reduced test volumes was also produced in order to allow the testing of SPE extracts at higher concentration factors.

SPE acetylcholinesterase inhibition assay

The acetylcholinesterase (AChE) screening of SPE extracts of field samples was conducted to evaluate the presence and bioavailablity of organophoshorus (OP) and carbamate pesticides. The assay quantifies AChE activity that is inhibited if exposure to these pesticides has occurred. The screen was conducted on extracts at different concentration factors (100, 33 and ten times original concentration) to allow the presence of very low concentrations to be detected. In order to screen small volume samples (allowing higher concentration factors) for AChE activity, the midge larva *Chironomus riparius* was used. Although this species is a sediment dweller it was chosen for this assay because it is easily maintained in culture and therefore provides a ready source of test animals which have not been previously exposed to contaminants. A methanol extract from the SPE column was reduced in volume by evaporation and then diluted with reconstituted bore-hole water to give the required concentration factor for testing.

For the AChE analysis, 1ml of methanol SPE extract was reconstituted in Burnham standard dilution water (reconstituted borehole water) providing a concentration factor of 100 times for the test solution. For the control, one millilitre of HPLC grade methanol was reconstituted in laboratory water. Preliminary trials indicated that ten-day-old Chironomids produced higher AChE levels and would therefore potentially allow a greater ability to discriminate inhibition. Ten *Chironomus riparius* lavae (ten days old) were exposed in ten millilitres of the reconstituted extract at different concentration factors. Chironomids were exposed in silanised glass beakers to reduce adsorption of toxicants to the test vessel. The Chironomids were not provided with synthetic sediment or food for the duration of the 24-hour exposure since this might result in loss of compounds from the water, or to enhanced uptake of compounds if the organisms ingest the substrate.

After the 24-hour exposure period the surviving Chironomids were sampled for AChE analysis as previously described, with the whole body being used to produce the sample homogenate. The homogenate was assayed for AChE activity and for reactivated AChE activity.

2.2.4 Deployment of *Gammarus pulex*

In order to monitor for the presence of transitory high concentrations of toxic compounds which may result in behavioural effects (eg.reduction in feeding rate) groups of 15 to 30 *Gammarus pulex* were placed at each site. Holding-chambers containing *Gammarus pulex* were placed at each of the main sites and controls. Individual *G. pulex* (mean length 10.3 mm) were placed in PVC tube sections with mesh ends. For assessment of feeding rate each animal was supplied with four conditioned maple (*Acer saccharum*) leaf discs (1.5cm diameter) which had been weighed dry and rehydrated four days prior to use. For mortality and enzyme inhibition studies animals were supplied with sufficient leaf material for the deployment period.

Gammarus held on site were also sampled in order to measure the activity of the enzyme acetylcholinesterase (AChE). Thirty animals were exposed for seven days, after which they were replaced by a new set of animals. After returning to the laboratory samples were prepared for AChE analysis as described in section 2.2.2. For each analysis ten *G. pulex* head capsules were pooled, producing three replicates for each of the study sites which were assessed.

2.2.5 Sediment bioassays

Some of the active substances applied in various pesticide formulations become strongly adsorbed to soil particles. During rainfall events these bound compounds have the potential to be transported in suspension in runoff water. Sediment dwelling organisms may become exposed to particulate adsorbed compounds once they settle out of suspension. In order to determine the presence of bioactive compounds in stream sediments, the sediment dwelling larvae of *Chironimus riparius* were introduced to sediment samples collected from sites along the Curtisden Green stream (Site C). Larval survival and growth, as well as emergence of larvae as adult Chironomids were recorded after a ten day exposure period in the sediment.

Samples of sediment from 6 sites on the Curtisden stream, plus the Cranbrook control stream, were taken with a hand-held grab on three occasions 26.03.98, 24.6.98 and 22.10.98 . The sediments were press-sieved with a 0.5mm mesh sieve to remove macro-invertebrates, coarse particles and detritus. The samples were frozen at -18°C. 5 days before the start of the test the samples were removed from the freezer and fresh egg ropes removed from the *Chironomus riparius* culture. Four days before the start of the test the sediment samples were decanted into beakers, the water was added and settling of disturbed fine sediment allowed to start (see the summary below for details). 1day prior to start, aeration of the containers was begun. On day zero the chironomids, now three days old, were counted out and added to the test vessels by wide mouthed pipette. The aeration was turned off for 24 hours upon addition of the larvae to allow settling of the animals. The first feeding occurred at this time and every other day thereafter. Pre-weighed quantities of finely ground tetramin was used as a supplemental larval feed. Tetramin was made into a suspension in water before addition to the test vessel. Physical readings of the test water were taken on days two and eight. At the end of the test the contents of the containers were washed through a 0.5 millimetre sieve. The animals were dried on paper towel and individual wet weights taken the first 11 chironomids (this number produced an optimal amount of tissue for the assay) taken at random were homogenised in preparation for the acetylcholine esterase assay (AChE) (Ellman *et al* 1961).

The summary of the sediment testing methodology used is given below. The method is based on the static water test with field collected sediment given in Environment Canada (1997), with three modifications. To suit the size of the beaker available the amount of sediment and water and the ratio between the two was increased to that given below (discretion in this matter is allowed in the prescribed method). The larger test vessels also allowed an increase in the number of animals per test container from ten to 20. This was done to ensure a sufficient number of survivors for the AChE assay to proceed. Finally, freezing of sediment to kill the indigenous fauna does not occur in the Environment Canada method. Freezing remains controversial for sediment storage (Burton 1991), due to release of soluble organic carbon and alteration of sediment structure. The sieving of the sample prior to freezing, however, deliberately homogenises the structure of the sediment, which in any case consists of several combined grab samples from thin layers of sediment rather than a core with preservable structure.

Many organophosphorus compounds are likely to become adsorbed to sediment particles, therefore benthic organisms may be exposed to them in the pore water or through ingestion of sediments. Therefore AChE activity of surviving organisms was also measured at the end of the test period.

Table 2.3 Summary of methodology used in sediment bioassay

Sediment depth	2 cm
Water Volume	600 ml
Container	1000 ml
Temperature	20°C+ or - 2°
Photoperiod	16 hr Light: 8hr Dark
Duration	10 days
Replicates	3/site
Test Water	Reconstituted Freshwater
Renewal Method	Static with replacement
Chironomids	20 2nd Instar per replicate
Feeding	0.8 –1.0 mg/larva/day Tetramin
Water Quality	D.O., pH, Temp, Conductivity on Days 0, 5, 10
Controls	Cranbrook, Acid Washed Sand
Sediment Characterisation	TOC, particle size spectrum
End Point	Wet wt, AChE inhibition, % survival.

2.2.6 Assessment of the indigenous macroinvertebrate population

Macroinvertebrates from the stream bed and edges were collected by kick sampling, a standard sampling technique employed by the Environment Agency in routine sampling programmes.

Drift net samplers were also deployed at each site and control. Animals moving actively, or passively drifting in the current were collected in a sampling pot tied to the end of a net bag fixed in mid-stream. The species composition of animals sampled in the drift was compared before and after high flow conditions and between main sites and associated controls. In this way change in the pattern of drifting organisms was compared with measured concentrations

of biologically active compounds in the stream water to determine if a relationship was apparent.

During 1997, 1998 and 1999 (weekly) drift-net samples were collected from sites A, C and D and the related control sites, as well as the control for the sheep-dip site. In addition nine to12 kick-samples were taken at roughly monthly intervals from each site and its control.

Calculation of species diversity as well as the biotic scoring system used by the Environment Agency (The Biological Monitoring Working Party or BMWP scoring system) were the two main methods used to compare sites identified in the present study. Data such as lists of species numbers is referred to as nominal scale data. Averages such as the mean or median cannot be applied in a meaningful way to such data. For this type of data, measures of 'diversity', that is the distribution of observations between categories can however be used. The diversity index used in this study is called the Shannon-Weiner diversity index (denoted by H'). The BMWP system provides a score for each macroinvertebrate family based on its sensitivity to organic pollution. The sum of these scores for a site produces the BMWP score. An additional value which is calculated is the average score for each of the taxa which receive a score in the BMWP system, this is the 'average score per taxon' or ASPT.

In addition to using the above univariate methods to describe the community data for each site a multivariate technique 'multi-dimensional scaling' or MDS was also used. MDS produces a two dimensional plot of the samples from the sites being compared. The plot is arranged in such a way that the rank order of the distances between samples on the plot agrees with the rank order of similarities between them. The statistical significance of the distances between samples can then be formally tested. The PRIMER statistics package (Clarke and Warwick, 1994) was used for all univariate and multivariate calculations.

In addition to the monthly kick samples and weekly drift-net samples at each site, a series of six duplicate kick samples were collected in the stream draining the Harpers Farm catchment (site C). Samples were taken between the spring-fed source to just above the point at which the stream joins the Lesser River Teise. A series of samples was collected during March, July and October 1998.

2.2.7 Assessment of the indigenous periphytic algal population

Diatom assemblages have often been used in water quality studies, most commonly as indicators of trophic status or organic pollution (Fritz *et al*. 1993, Christie and Smol 1993, Lange-Bertalot 1979). These studies concentrated initially on static water bodies, where the accumulation of diatom valves on the surface of lake sediments for example, exhibited characteristic temporal patterns. In recent years, however, studies have explored the relationship between habitat, and other environmental variables, and diatom distribution (Pan *et al*. 1996, Kutka and Richards 1996). This allows data from stream systems in which there may large fluctuations in physical and chemical parameters to be used with greater understanding. River water-monitoring studies have concentrated on trophic status (Round 1991); biological indices being developed with organic pollution in mind (Kelly *et al*. 1995).

Diatoms are studied because they are pollution sensitive (Pan *et al*. 1996) and because the epilithic and epiphytic forms (attached to stones and plants, respectively), attach securely to

artificial substrates, such as glass slides or unglazed tiles, allowing control of colonisation period, habitat type and sampling area.

The feasibility of using glass slide substrates with direct examination of attached algal communities as a means of monitoring community change associated with pesticides in surface runoff was therefore evaluated.
Artificial substrates (glass microscope slides) were deployed in one of the stream sites in order to make an assessment of the number and species of periphytic stream algae (those attached to surfaces) present at the chosen site.

Table 2. Bioassay procedures used in the analysis of water and sediment samples at each of the sites

Technique	Method of use	Site at which technique used
Mortality assessment	Daphnia magna exposed for 48 hours to water samples collected during storm events	Site A, C and D
	Daphnia magna exposed for 48 hours to SPE extracts	Site A, C and D samples for B taken not tested
	Gammarus pulex placed in cages at site, mortality assessed every 7 days	Site A, C and D
Feeding rate bioassay	*Gammarus pulex* deployed at site for 1-2 weeks. Consumption of leaf material measured	Site C and D for period of 63 days
Acetylcholinesterase inhibition assay	*Gammarus pulex* placed at site for periods of 1-2 weeks, AChE activity measured	Site C
	Gammarus exposed for 24 hours to water samples collected from site, AChE activity measured	Site C
	Chironomus riparius exposed for 24hours to SPE extracts, AChE activity measured.	Site C
	Chironomus riparius exposed to sediment from site for ten day period and AChE activity measured	Site C only
Chironomid growth assay	*Chironomus riparius* exposed to sediment from site for ten day period and growth measured	Site C only
Algal growth assay	Algae grown in water from site collected by automatic sampler during storm events	Site A, C and D
	Algae grown in diluted solvent extract from SPE column	Site A, C and D
Lemna minor conductivity assay	48 hour exposure of *Lemna* minor to diluted solvent extract from SPE column	Site A, C and D
Sampling of indigenous invertebrates	Both kick samples and drift samples (except site B for which kick only)	Site A, B, C and D Site C also had a series of samples taken along its length
Sampling of indigenous algae	glass slides placed in stream sampled after weekly intervals	Site A, C and D

3. RESULTS

3.1 Site B – Sheep site

3.1.1 Estimation of the disposal intensity of sheep-dip at Site B

An aerial survey was conducted during August 1998 at Site B in order to establish the numbers of sheep grazing in the immediate catchment of the River Tillingham. The river and its main tributary streams are estimated to drain an area of 15 km^2. The aerial survey covered a larger area of 21 km^2 since it was considered that spent dip from animals treated in this area and which was disposed of locally, may ultimately be disposed of within the River Tillingham catchment. Several owners of flocks in the catchment had also confirmed that dip disposal takes place on their farms. Based on aerial photographs taken during August 1999, the estimated number of sheep in the survey area was 4000 animals. Data collected by MAFF statistics division for surveys conducted during June 1999 indicated that 7100 sheep (including lambs) were owned by farms in the catchment of the River Tillingham, upstream of the sampling point. The number of sheep counted in the aerial survey was used to estimate dip disposal in the catchment since owners of flocks may not graze all animals within the catchment area. Generally two dips a year are applied with 5 litres of diluted dip formulation per animal (Blackmore and Clark, 1994). Based on two dip applications of 5 litres to each of 4000 animals, 20000 litres of spent diluted dip could potentially be produced. This total is probably divided amongst several dipping operations and hence disposed of at several different sites. Recommended disposal rate of spent dip to land is 5 M^3/ha (MAFF, 1998). Dip formulations with organophosphorus insecticides (OPs) as the active substance typically use five to 60% a.s./litre of formulation, those with synthetic pyrethroids (SPs) as active substance use two to six% a.s./litre of formulation. Using the maximum active substance values an estimated 12000 litres of OP (most probably diazinon, or propetamphos) or 1200 litres of SP (most probably cypermethrin or flumethrin) could be disposed of in the immediate catchment. Dipping of sheep generally occurs in late summer early autumn. Dip disposal will also usually take place during this period.

Automatic samples were taken from the River Tillingham between November 1998 to January 1999 following the first major storm events that took place after the period during which dip disposal would have occurred. The OP compounds diazinon and propetamphos were analysed for but not detected in any of samples during this period (detection limit <0.05 µg/L). The SP compound cypermethrin was also analysed for but was not detected in any of the samples (detection limit <0.13 µg/L).

Due to the presence of an Environment Agency flow-gauging site downstream of the water sampling point it was not possible to deploy caged organisms or a drift net at the site. Nevertheless kick samples were collected at the site at monthly intervals. The sampler triggered on three occasions between the 6.11.98 to 8.12.98 however, due to a software problem with the logging unit, automatic samples were not taken. Several samples collected during this period were nevertheless bulked for analysis. Macroinvertebrates were collected from the stream by kick sampling on the 2.12.98 during a subsequent storm event, and thereafter at approximately monthly intervals. Figure 3.1 shows the sequence of triggered sampling events during December 1998. Two events were sampled during December and a

further four occurred during January of which two were sampled. The December events at Site B are shown in Table 3.1.

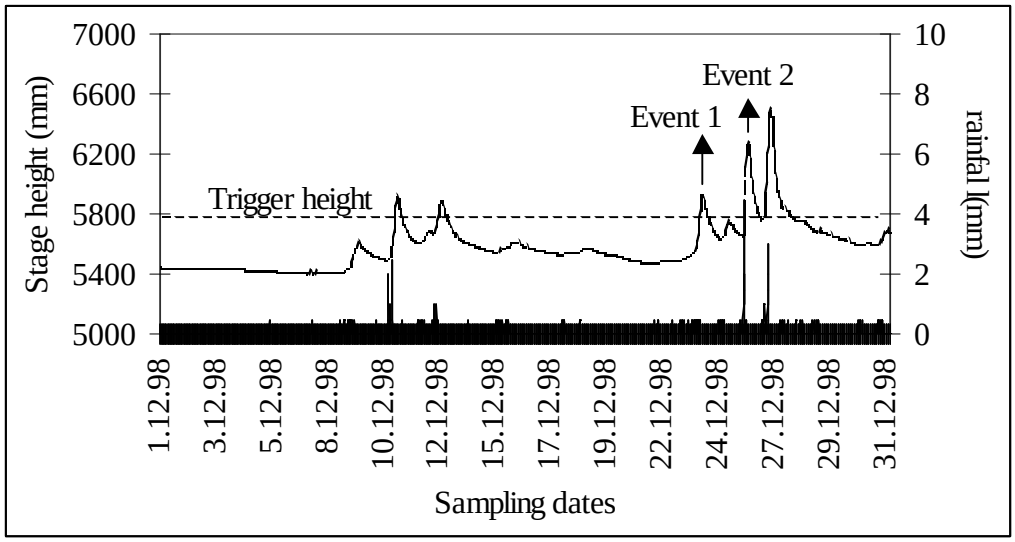

Figure 3.1 Storm events that triggered the automatic sampler during December 1998

Table 3.1 Bioassay procedures and chemical analysis for samples collected between winter 1998 and spring 1999 at site B.

Sample date	Storm event	Daphnia bioassay	sample for Invertebrates	sample for AChE	sample for SPE	chemical analysis
5.11 to 11.11.98						√
17.11 to 23.11.98	√					√
2.12.98 to 8.12.98	√	√	√	√	√	√
16.12.98		√		√	√	
23.12.98	√	√		√	√	√
25.12.98	√					√
6.1.99	√	√	√	√	√	
12.1.99	√					√
16.1.99	√					√
27.1.99	√	√		√	√	
3.2.99			√			
2.3.99	√	√	√	√	√	
17.3.99			√			
29.3.99			√			
21.4.99	√	√		√	√	
27.4.99	√		√			
5.5.99		√				
3.6.99	√				√	
9.6.99	√				√	

The *Daphnia magna* bioassay was run directly on water samples collected from Site B. There were no instances of immobilisation of animals in samples collected from storm events between December 1998 and May 1999.

Kick samples collected at the time of the first winter storm events at this site and also in January showed moderate numbers of invertebrate groups which are known to be particularly sensitive to insecticide exposure: *Gammarus pulex*, the cased caddisfly larvae (Hydropsychidae), mayfly larvae (Baetidae,Ephemeridae and Leptophlebidae) stonefly larvae (Nemouridae) and damselfly larvae (Zygoptera) were all sampled. Both samples were comparable in species composition with the exception that no hydropsychidae were sampled in January and *Gammarus* numbers had reduced. The BMWP and species diversity scores for each sample are shown in Table 3.2. Since pesticides were not measured in the water samples during these periods this difference may result from seasonal factors such as reduced water temperature or the high number of storm events.

Table 3.2 Biotic indices calculated for samples collected during the main period of storm events at Site B, following the period in the latter part of summer early autumn when most dip disposal was likely to occur.

Community measure	2.12.98	6.1.99
BMWP score	74	75
ASPT score	4.9	5.4
Species Richness (Margalef)	3.0	3.1
Shannon-Weiner index	1.8	1.4
Eveness index (Pielou)	0.6	0.5
Total number individuals	300	187
Total number of species	16	16

3.2 Site A

The major groups of compounds applied at Site A between spring 1997 and spring 1999 were herbicides and fungicides. The actual applications made are shown in Table 3.3. Particularly wet weather during autumn/winter 1998 to 1999 delayed and in some cases prevented application of insecticides.

Table 3.3 Pesticide usage data for the study catchment of Site A arable, Applications between spring 1997 and spring 1999.

(1) compounds followed by '(a)' analysed for in samples, and those followed by '(d)' detected in samples

Sample site	Application date * number applications		Pesticide type	Active substance(1)
(A) Rye Farm near Colchester (Abberton catchment)	1997	Mar(1)*	fungicide	**fenpropimorph (a)**
		May(1)		+tridemorph
		Apr(1)May(1)	fungicide	flusilazole
		Sept	herbicide	metazachlor
	1998	March	fungicide	**tebuconazole (a)**
		April	insecticide	lambda-cyhalothrin
	1999	January	herbicide	isoproturon
			herbicide	**diflufenican (a)**
Friday wood Farm near Colchester (Abberton catchment)	1997	February	herbicide	terbutryn+trietazine
			herbicide	**simazine (d)**
			herbicide	fluazifop-P
			aphicide	pirimicarb
			herbicide	cycloxydim
			fungicide	**fenpropimorph (a)**
			fungicide	chlorothalonil
			herbicide	tralkoxydim
			herbicide	metazachlor
			herbicide	clopyralid
			fungicide	tebuconazole
	1998	Feb/Mar	herbicide	isoproturon
			herbicide	**diflufenican**+isoproturon**(d)**
			insecticide	deltamethrin
		April	herbicide	tralkoxydim
		May	herbicide	fluoroxpyr
		June	fungicide	tebuconazole
			fungicide	**fenpropimorph(a)**
			herbicide	metsulfuron-methyl
		September	herbicide	metazachlor
		December	herbicide	isoproturon
			herbicide	diflufenican
				fluazifop-P-butyl
	1999	February	herbicide	cyanazine
		March	fungicide	**tebuconazole (d)**
		April	herbicide	clodinafop-propargyl
			herbicide	tralkoxydim

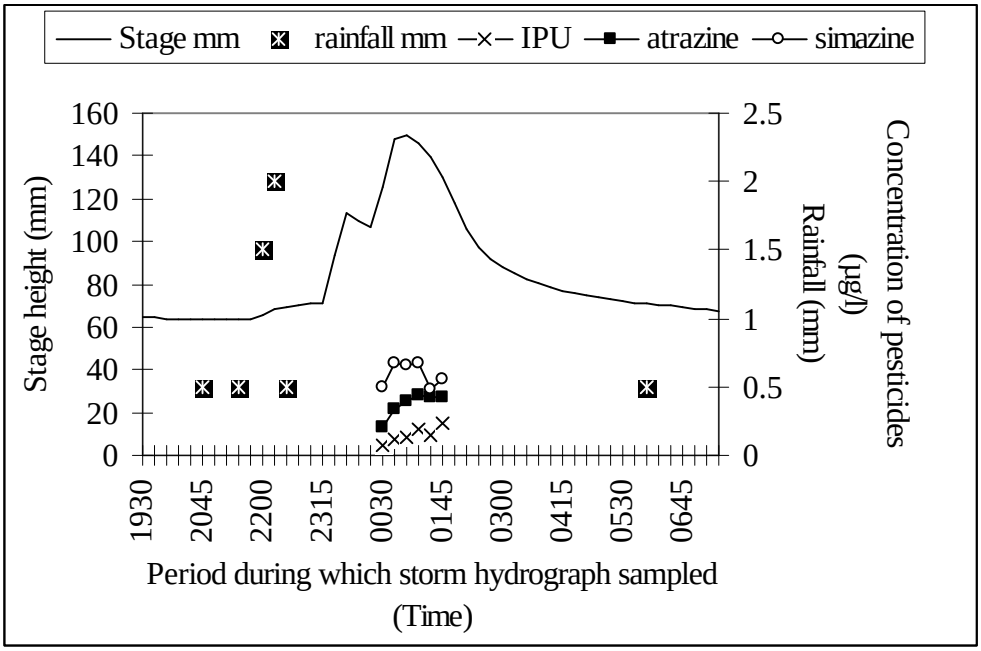

Figure 3.2 Concentration of pesticides (µg/l) sampled between the 9.5.97 to 10.5.97 in the stream draining the Site A catchment.

Figure 3.2 shows a typical storm hydrograph for Site A. The stream on this site was characterised by peaks of very short duration. The event shown lasts for four hours. During this event three herbicides, isoproturon, atrazine and simazine, were detected.

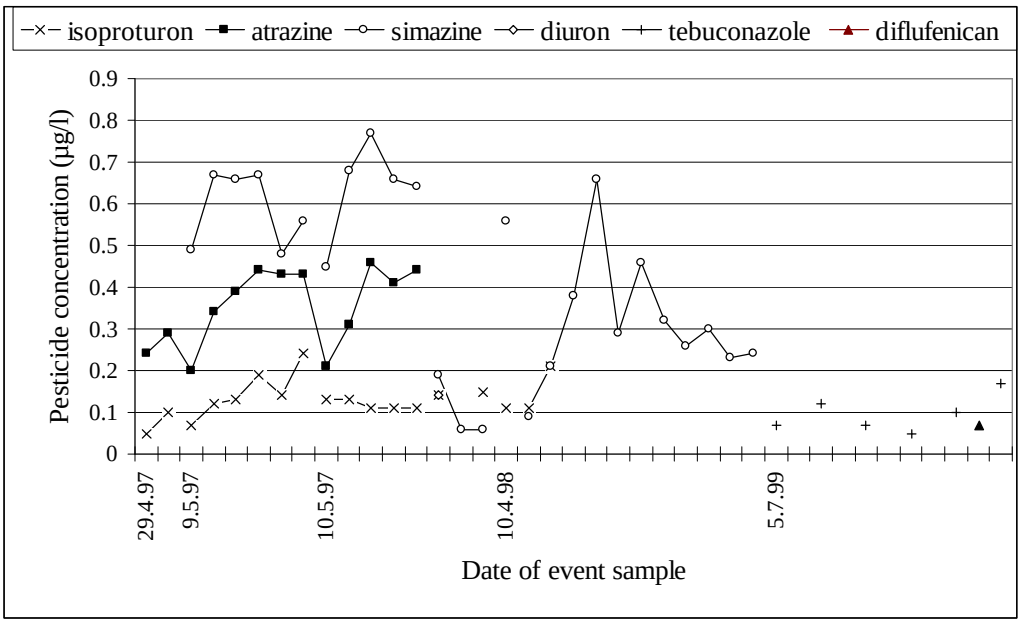

Figure 3.3 Concentration of pesticides (µg/l) sampled in all storm events between the 29.4.97 to 5.7.99 in the stream draining the Site A catchment. Note that samples taken on 12.3.98, 10.4.98 and the first sample on 15.4.98 were all taken during normal conditions of flow.

Figure 3.3 shows the concentration of different pesticides measured during storm events at Site A. The concentration of those compounds measured is less than 1µg/l in each case. Simazine was the most consistently detected compound.

A range of bioassay procedures were applied to samples collected during storm events at Site A. Table 3.4 shows the procedures applied to samples taken on specific dates.

Table 3.4 Bioassay procedures conducted for samples collected between winter 1998 and spring 1999 at site A.

Sample date	Storm event	Daphnia bioassay	Algal bioassay	sample for Invertebrates	sample for AChE	sample for SPE
10.11.97	√	√	√		√	
3.12.97	√	√	√		√	√
22.12.97	√	√	√		√	√
7.1.98	√	√	√		√	√
15.1.98				√		
22.1.98			√			
28.1.98			√			
5.2.98			√			
12.2.98			√	√		
19.2.98			√			
5.3.98						
12.3.98				√		
16.4.98	√		√	√	√	√
23.4.98			√			
7.5.98			√			
14.5.98			√	√		
21.5.98			√			
5.8.98				√		
16.9.98						
4.11.98				√		
3.12.98				√		
7.1.99				√		
4.2.99	√			√		√
3.3.99				√		
11.3.99	√					√
30.3.99				√		
28.4.99				√		
26.5.99				√		

Gammarus pulex mortality was assessed in animals caged at site and drift net samples of macroinvertebrates were collected during each week of the study from Site A and its control stream Birch Brook, this is not shown in Table 3.4. Glass slides were also placed at Site A and its control in order to sample some of the periphytic algal population in the stream.

3.2.1 Direct toxicity testing

No mortality was recorded in any of the direct toxicity tests of water from automatically sampled events at Site D, using the crustacean *Daphnia magna.*

Gammarus pulex held in cages in the stream exhibited relatively high mortality of 13 to 26% between January and June of 1998. This was thought to be due to the continual settlement of sediment in the cages, in particular during a storm event on the 16.04.98, when 53% mortality resulted. Cages were only deployed until the beginning of June 1998 at this site due to the sedimentation problems.

In addition to running animal bioassays, the unicellular green alga *Raphidocellis subcapitata* was grown in samples collected from each site during periods of increased flow. Growth of algae in samples collected at site A are shown in Figure 3.4. Consecutive stream water samples collected at hourly intervals over a 24- hour period were bulked to produce four samples: 1 to 6 hours, 7 to 12 hours, 13 to 17 hours and 18 to 24 hours. In some cases less than 24 samples were collected, in these instances bulk samples were made up of fewer than six samples and/or fewer than four bulk samples were collected. Due to the flow characteristics of the headwater streams being studied, only the first sample batch was likely to contain pesticides therefore the second to fourth samples were used to provide a comparison to indicate if growth inhibition was occurring. To ensure that growth of algae was not limited by lack of nutrients, selected nutrients were added to each bulk sample. Sub-samples of each bulk sample were also combined prior to the addition of extra nutrients in order to produce a sample in which the growth of *Raphidocellis* could be related to the concentration of nutrients present in
the original samples.

The number of algae increased from 50,000 to between 4 to 6 million cells in 96 hours in many of the samples. At site A growth of algae in the sample which had no added nutrients was comparable with that in the nutrient spiked samples (Figure 3.4), which indicated that growth was not nutrient limited at this site during this period. It was apparent that growth was much reduced in some groups of bulk samples. Reduced growth in some of the samples within a batch from at least one event may have been due to the presence of higher concentrations of phytoactive compounds. This was noted at site A in early winter 1997 and also in samples from late spring 1998. Reference to Figure 3.3 shows that simazine diuron and isoproturon were present a low concentrations (<0.2 µg/l) in a sample collected from Site A in December. It is possible that these compounds were present at higher concentrations during November when reduced algal growth was observed. It should also be noted however, that since no applications of any of these compounds were recorded during the late summer or autumn these low levels might represent residual concentrations from much earlier applications. The reduced algal growth observed in the sample from 16.4.98 occurred in samples that contained simazine and isoproturon but at maximal concentrations of only 0.2 to 0.6µg/l respectively. A summary of the toxicity of a range of pesticides detected at each of the sites is shown in Table 3.5. It can be seen by reference to the algal toxicity data in Table 3.5 that even the combined concentration of herbicides detected in the water samples collected at Site A in April 1998, are unlikely to have produced the observed algal growth inhibition.

Table 3.5. Toxicity data (µg/l) for a range of animal and plant groups for the pesticides detected at each of the sites during 1997. Exposure time is stated at the top of the column or in brackets after the LC/EC50 value. Toxicity values derived from Ref. 19 unless otherwise listed. Environmental Quality Standard (EQS) values obtained from * Ref 1 - 3 (upper Figure is the recommended annual average concentration, the lower value is the maximum allowable concentration.) Toxicity figures used were the lowest found in the literature.

Pesticide	**EC_{50} Green Algae**	**EC_{50} Diatom**	**EC_{50} Macrophyte**	**EC_{50} Daphnia 48 h**	**LC_{50} *Gammarus* sp.**	**LC_{50} insect**	**LC_{50} Fish 96 h**	**EQS (Ref 1-3)**
Atrazine	21(4d) (Ref 4)	-	56 (10d) (Ref 4)	6900	5700 (Ref 5)	720 (Ref5)	4300	2 10
Chlorotoluron	24(3d) (Ref 3)	-	-	67,000	-	-	35,000	2 20 *
2,4-D	88,900 (1d) (Ref 6)	2900 (1d) (Ref 7)	10 - 100 (11d) (Ref 8)	4000-100000 (Ref 8)	100000 (Ref 9)	-	1,100 (48h)	20 200
Diuron	36 (3d) (Ref 10)	93 (3d) (Ref 11)	41(7d) (Ref 12)	12,000	380-1800 (Ref 13)	2800 (Ref 14)	5,600	2 20
Fenpropimorph	-	-	-	2400	-	-	3,200	-
Isoproturon	21(4d) (Ref 4)	-	31(10d) (Ref 4)	507,000	-	-	37,000	2 20
MCPA	1,400 (1d) (Ref 7)	2,000(3d) (Ref 7)	1,400 (7d) (Ref 7)	>100,000	-	-	232,000	-
MCPB	57,000 (24d) (Ref 15)	-	-	3,300	-	-	11,000	-
Mecoprop	102,700 (4d) (Ref 4)	-	7,350 (10d) (Ref 4)	420,000	-	-	150,000 to 220,000	20 200
Propiconazole	5,000 (3d) (Ref 16)	3,300 (6d) (Ref 16)	9,020 (14) (Ref 11)	4,800-11500	-	-	5,300	-
Simazine	73(1d) (Ref 16)	90 (5d) (Ref 15)	150 (1d) (Ref 1)	1000 (Ref 1)	21000 (Ref 8)	-	49,000	2 10

References Table 3.5:

1 Proposed Environmental Quality Standards for atrazine and simazine in water
2 Proposed Environmental Quality Standards for 2,4-D and mecoprop in water
3 Proposed Environmental Quality Standards for diuron, linuron, chlorotoluron and isoproturon in water
4 Kirby, M. and Sheahan, D.A. (1994).
5 Macek K.J., Buxton K.S., Sauter S., Gnilka S. and Dean J.W. 1976.
6 Faust,M., R.Altenburger, W.Boedeker, and L.H.Grimme 1993
7 Peterson, H.G., C.Boutin, P.A. Martin, K.E. Freemark, N.J.Ruecker, and M.J.Moody 1994.
8 Schott,C.D. and E.C.Worthley 1974.
9 Sanders, H.O.1970.
10 Schafer,H., H.Hettler, U.Fritsche, G.Pitzen, G.Roderer, and A.Wenzel 1994
11 Office of Pesticide Programs 1995 Environmental Effects Database (EEDB)
12 Liu, L.C. and A.Cendeno-Maldonado 1974.
13 Sanders H.O. 1969.
14 Sanders H.O. and Cope O.B. 1968.
15 Kirkwood,R.C. and W.W.Fletcher 1970
16 Kallqvist,T. and R.Romstad 1994

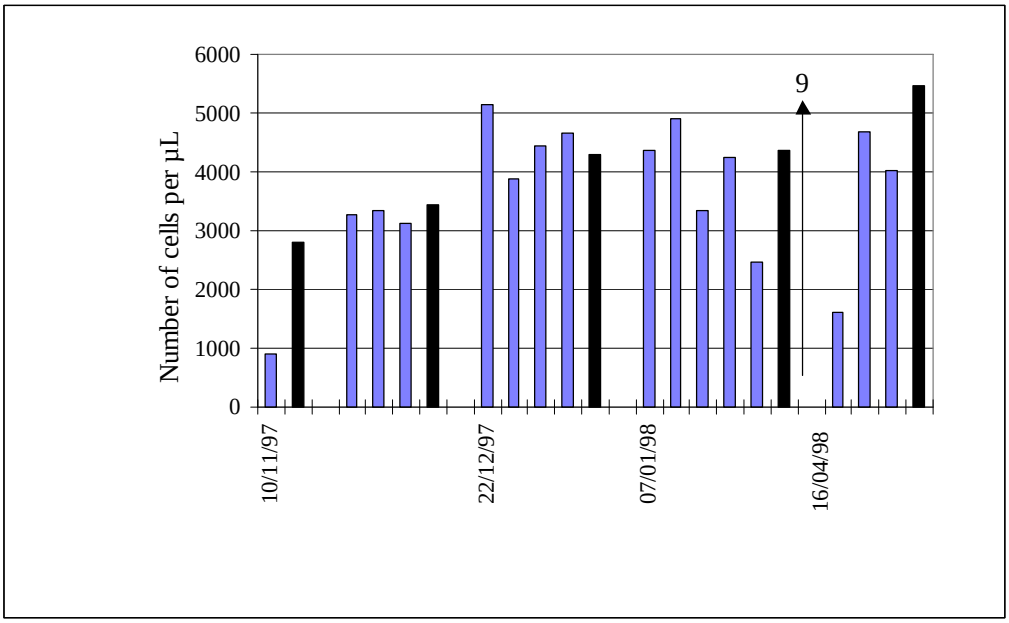

Figure 3.4 Growth of *Raphidocellis subcapitata* in bulk water samples collected from the stream at site A during periods of increased flow. The black bars denote samples from a batch that did not have extra nutrients added. An arrow indicates the cumulative number of pesticide applications occurring between 10/11/97 and 16/4/98.

Samples taken on each date represent composites of hourly samples collected over consecutive 6 to 8 hour intervals. Pesticide concentrations were generally higher in the first part of a storm event therefore the first bulk sample would be expected to produce the largest inhibition of algal growth. The unspiked samples in these groups are composites of all samples taken prior to spiking with nutrients. The sample taken on the 10.11.97 was divided into two equal volumes one of which was spiked and one, which remained unspiked. The reduced algal growth, which was observed in the spiked sample on this date, is therefore difficult to explain since equal concentrations of pesticides would be expected in both the

spiked and unspiked sample. For all storm event samples the pesticides detected, mainly herbicides, were not present at sufficient concentrations to explain the level of algal growth inhibition observed in some of the samples. This suggests that other toxic compounds, which were not measured, were also present in the samples.

3.2.2 Toxicity testing of concentrated extracts from solid-phase columns

Available information on pesticides applied at this site indicated that they were all of low toxicity to *Daphnia magna* (based on laboratory data). This was supported by the fact that none of the water samples tested directly for toxicity showed evidence of effects in the *Daphnia magna* immobilisation assay. In addition SPE samples collected from storm events on the 7.1.98 and 11.3.99 which were tested using the *Daphnia magna* bioassay showed no toxic effects at a concentration factor of 100x. The main focus of the SPE work was therefore on phytotoxic effects. Figure 3.5 shows a comparison of algal growth in an SPE sample collected during a storm event on 16.4.98 and during a period of relatively low flow on the 16.9.98.

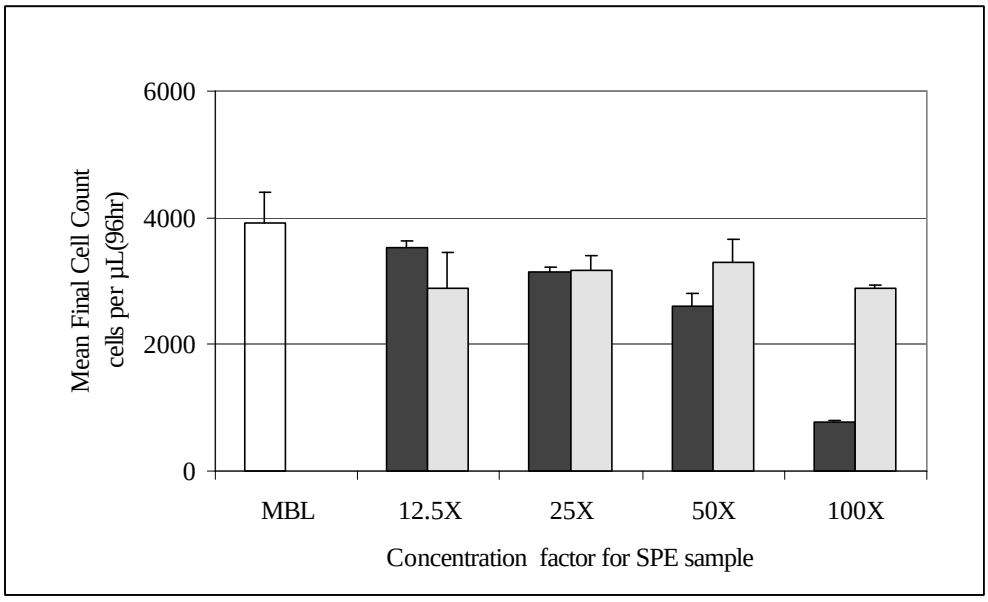

Figure 3.5 Growth of *Raphidocellis subcapitata* in SPE samples collected from the stream at site A during a period of increased flow on 16.4.98 (black bars) and in an SPE sample collected during a period of low flow, 16.9.98 (light bars). Growth in MBL medium alone is denoted by the hatched bar. All samples except the 12.5% event sample produced significantly lower growth than the control, $p<0.05$

The herbicide simazine was the only pesticide detected in samples on the 16.4.98 and was present at an average concentration of 0.33µg/l. At a concentartion factor of 100 this would be equivalent to a concentration of 33µg/l, the 24 hour EC50 for growth of *Raphidocellis* was indicated to be 73µg/l (Faust *et. al.,* 1993), and hence the marked reduction in growth was probably a result of exposure to simazine. It should be noted that during a period of low flow in September 1998, when herbicides were unlikely to be present as a result of field drainage, algal growth was also slightly reduced in SPE samples.

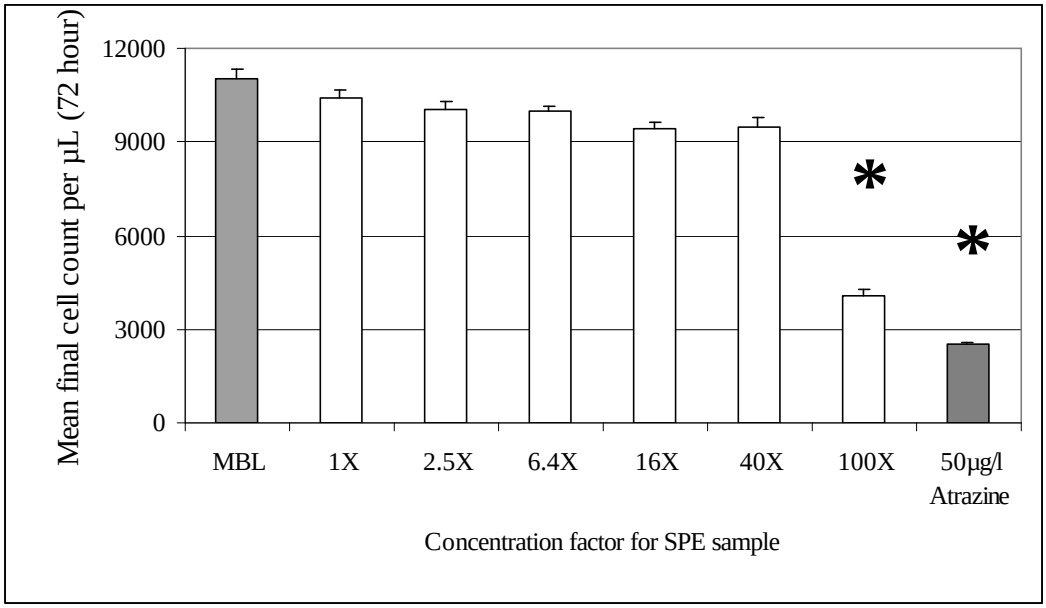

Figure 3.6 Mean (95 percent confidence limits) growth of *Raphidocellis subcapitata* in SPE samples collected from the stream at site A during a period of increased flow on 11.3.99 (black bars) and in an atrazine stock solution (light bar). Growth in MBL medium alone is denoted by the hatched bar.
*** denotes that the cell growth was significantly reduced in these treatments relative to all others and the control ($p<0.05$)**

On a subsequent occasion, 11.3.99 an SPE sample was tested on *Raphidocellis* cultures grown in the wells of a plate reader (Figure 3.6). The modified assay procedure used a higher initial inoculation density ($1x10^6$ cells per ml), and the test was terminated after 72 hours. Growth inhibition is apparent in the 100x concentration SPE sample but not at lower concentration factors. The level of inhibition is comparable to that in a 50µg/l atrazine solution. Of the pesticides analysed only tebuconazole was detected at a mean measured concentration 0.1µg/l. Tebuconazole is a fungicide, for which, available data does not indicate herbicidal properties. The likelihood however is that herbicides were also present in the samples.

The response of the macrophyte *Lemna minor* in the conductivity bioassay of SPE extracts from Site A on the 11.3.99 is shown in Figure 3.7. When paraquat is added to all samples in the bioassay of SPE extracts it produces a rise in conductivity. Samples that also contain photosynthesis inhibiting herbicides produce a reduction in conductivity as compared to that produced by paraquat alone. The magnitude of the difference between conductivity levels in the paraquat only treatment and those treatments with added SPE extract, is proportional to the concentration of photosynthesis inhibiting herbicides present. Although conductivity appears to decrease slightly with increased sample concentration factor, only at a concentration factor of 100x is the mean conductivity significantly less than that of the paraquat treatment. If it is assumed that the herbicidal activity was due to atrazine alone this would be equivalent to an atrazine concentration of approximately100µg/l in the 100x SPE sample. This would indicate a raw water concentration of very approximately 1 µg/L of atrazine or other herbicides with a similar mode of action.

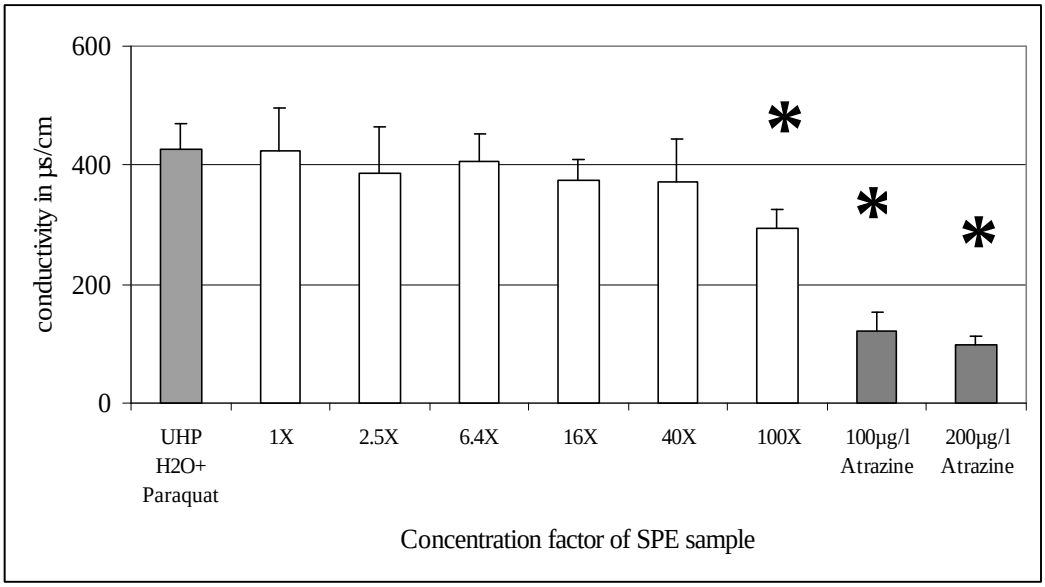

Figure 3.7 Mean (95 percent confidence limits) increase in conductivity over 48 hours in *Lemna minor* bioassay of SPE extract of a stream water sample collected during a period of increased stream-flow at site A (11.3.99). The effect of two different concentrations of atrazine on the assay response is also included. (*=significantly different from all other treatments $p<0.05$)

3.2.3 Predicted toxicity in water samples

The toxicity data in Table 3.5, together with the measured concentration of individual compounds detected at each of the sites, were used to predict the toxicity of stream water samples: The measured concentration of each compound was converted to toxic units. Toxic units are calculated by dividing the concentration of a compound present in a sample by that concentration which produces a specified toxic effect to a named organism after a standard exposure time. If 50µg/l of compound 'A', produces a 50% immobilisation of *Daphnia magna* after 48 hours exposure in the laboratory and 50µg/l of the same compound is measured in a stream water sample, it can be said that there is 1 unit of toxicity in the sample. If there were only 25µg/L of compound 'A' in the water sample this would represent 0.5 toxic units (TU). Generally the concentration of a compound present in an environmental sample represents a fraction of that necessary to cause acute toxic effects and hence less than one toxic unit of a compound is present. Compounds present at a level below which acute toxic effects are observed may nevertheless produce effects of significance over longer exposure periods. By determining how far below the threshold for acute toxic effects an environmental sample falls, it is possible to identify those samples for which there is a high probability of chronic toxicological effects. When water samples from particular sites regularly exceed a pre-defined level at which chronic effects may be expected to occur, the sites in question may be considered to be at high risk of degradation in biological quality.

It is assumed in this assessment that the toxic effects of the majority of compounds are additive. Therefore toxic units or fractions of toxic units of compounds are added. Summed toxic units can then form the basis of predictions as to whether toxic effects are likely to occur in a particular situation where mixtures of chemicals are present.

Acute toxic effects rarely result from exposure of organisms to surface waters unless they are heavily contaminated or contain transient high concentrations of compounds from diffuse sources. However the concentration of compounds present in a sample can be increased by concentration using solid-phase extraction. The sum of toxic units for compounds detected in a raw water sample can be compared with the toxicity of sample extracts from SPE columns. When samples are more toxic than predicted on the basis of measured compounds the presence of other as yet unidentified compounds is indicated. The use of SPE therefore is a way of estimating the potential of a sample to cause sublethal toxicity, even though the bioassay procedure may only give a lethal endpoint

Figure 3.8 shows the calculated toxic unit values to *Daphnia magna* for pesticides detected in water samples collected during storm events at site A, between May 1997 and April 1998.

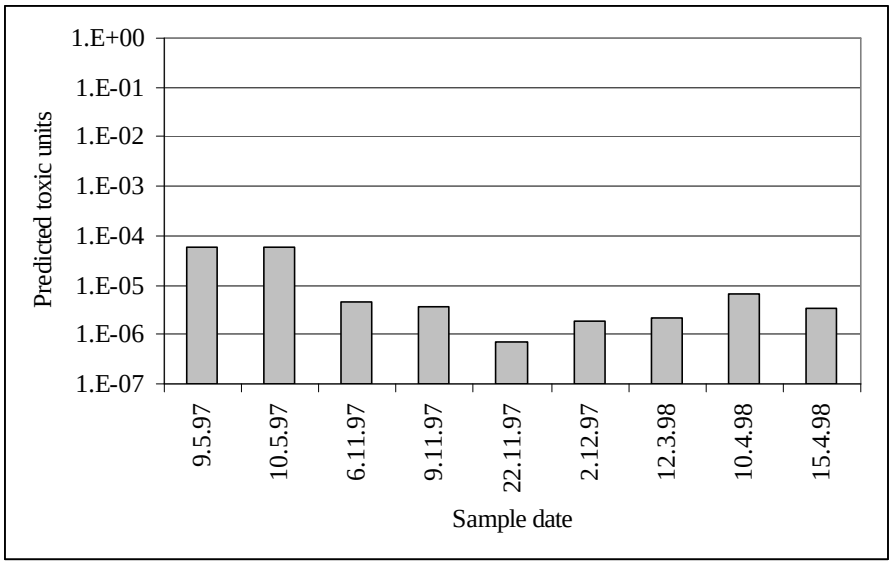

Figure 3.8 Graph showing predicted toxic units (grey bars*) for *Daphnia magna* based on compounds detected in water samples collected during increased stream-flow, following rainfall at site A.

***To calculate the toxic units, the concentration of each compound measured in a water sample was divided by the relevant toxicity figure for *Daphnia magna* from Table 3.5 and these values were then summed.**

During three of the nine events sampled, a concentration factor of <100x would be required to produce one unit of toxicity. Measured algal inhibition in SPE samples for two of the events are also shown in Figure 3.8. Actual growth inhibition is greater than predicted from the measured compounds. This may indicate that other unmeasured compounds are also having an effect. It is particularly likely for the sample taken on the 11.3.99 that other toxic compounds were present, since the only compound analysed which was detected, was the fungicide tebuconazole which is of low phytotoxicity.

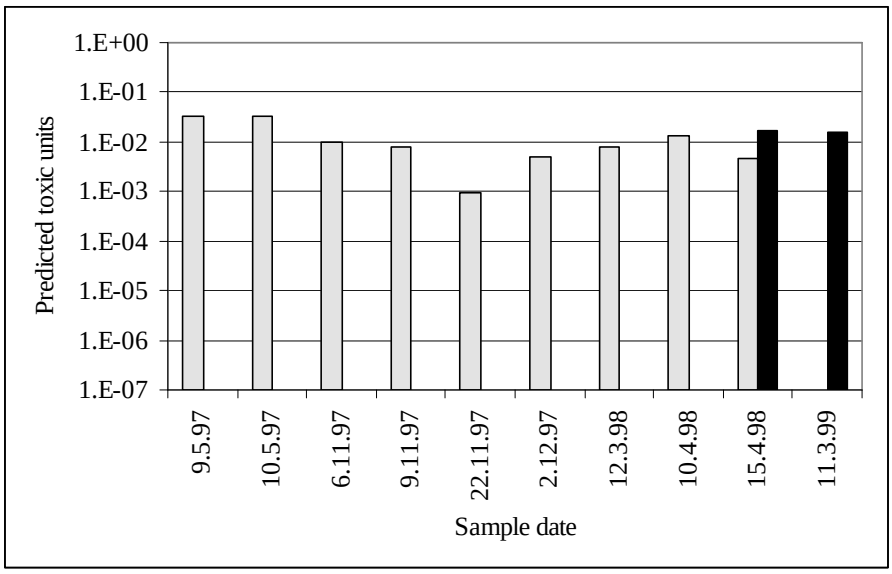

Figure 3.9 Graph showing predicted toxic units(grey bars*) for the unicellular green algae *Raphidocellis subcapitatum* based on compounds detected in water samples collected during increased stream-flow, following rainfall at site A. Measured algal growth inhibition in SPE samples is also indicated (black bars).

***To calculate the toxic units, the concentration of each compound measured in a water sample was divided by the relevant toxicity figure for unicellular algae from Table 3.5 and these values were then summed.**

3.2 Site C

3.3.1 Pesticide application and storm event data

Table 3.6 Pesticide usage data for Harpers Farm study catchment, Site C (Hops & Apples) (1) compounds followed by '(a)' analysed for in samples, and those followed by '(d)' detected in samples

Sample site	Application date * (number applications)		Pesticide type	Active substance (1)
(C) Harpers Farm near Tunbridge (Hop site)	1997:	Mar (1)*	herbicide	**simazine(d)**
		Mar(1)	herbicide	diquat+paraquat
		Mar(1)	herbicide	triclopyr
		Mar(1) Apr(2) May(1) Jun(1) Jul(1) Aug(1)	fungicide	copper oxychloride+ **metalaxyl(a)**
		Mar(1) Apr(2)	fungicide	**pyrazophos(a)**
		May(3) Jun(2) Jul(3)	fungicide	**penconazole(a)**
		May(2) Jun(1)	fungicide	fosetyl al salt
		May(1) Jul(1)	insecticide	imidacloprid
		May(3) Jun(2) Jul(3) Aug(1)	fungicide	bupirimate
		Jun(2) Jul(1)	insecticide	tebufenpyrad
		Jun(2) Jul(1)	insecticide	clofentezine
		Aug(1)	fungicide	myclobutanil
	1998	March(1) April(1)May(1) June(2) July(4)	fungicide	copper oxychloride+ metalaxyl
		March(1) April(1) May(1)	fungicide	pyrazophos
		May(1) June(2)	fungicide	fosetyl al salt
		May(2) June(2) July(1)	fungicide	bupirimate
		August(1)	fungicide	penconazole
		June(2)	insecticide	tebufenpyrad
		June(2)	insecticide	clofentezine
	1999	February(1)	herbicide	simazine
		February(1)	herbicide	diuron
		February(1)	herbicide	glyphosate
		February(1) April(1)	fungicide	copper oxychloride
		February(1)	fungicide	pyrazophos
		April(1)	fungicide	fosetyl al salt
		April(1)	fungicide	**penconazole(a)**
(C) Harpers Farm nr Tunbridge (Orchard site)	1997	Mar(2) Apr(2) May(3)	fungicide	dithianon
		Apr(1)	herbicide	glyphosate
		Apr(1)	herbicide	**simazine(d)**
		Apr(1)	herbicide	**diuron(d)**
		Apr(1) Jun(1)	fungicide	captan
		Apr(3) May(3) Jun(1)	herbicide	chlorpropham
		Apr(1) Jun(1)	insecticide	**chlorpyrifos(a)**
	1998	February	herbicide	triclopyr,**simazine**,**diuron(d)**
		March(2)April(3)June(3)July(1)	fungicide	dithianon
		April(2) May(1) June(1)July(3)	fungicide	pyrifenox
		April(1) June(2)	insecticide	**chlorpyrifos(a)**
		May(2) June(4)July(3)	fungicide	**bupirimate(a)**
		May(2) June(3)	fungicide	**penconazole(a)**
		May(2) June(3) July(3)	fungicide	paclobutazol
	1999	April(1)	fungicide	kresoxim-methyl
		April(1)	fungicide	dithianon, **penconazole(a)**
		April(2)	insecticide fungicide	chlorpyrifos, pyrifenox

A wide range of pesticides were applied at Site C due to the presence of a mixture of agricultural land use in the catchment, including orchards, hop gardens and soft fruit cultivation.
Over the three year study period however a limited range of compounds were detected in the stream draining the site. The compounds detected were herbicides, simazine, diuron, atrazine chlorotoluron and isoproturon. There was no application record for isoproturon for either the orchards or hop-gardens but it is a frequently used herbicide on cereal crops and there was an area of approximately 17 Ha at the top of the catchment on which winter wheat was grown during the study.

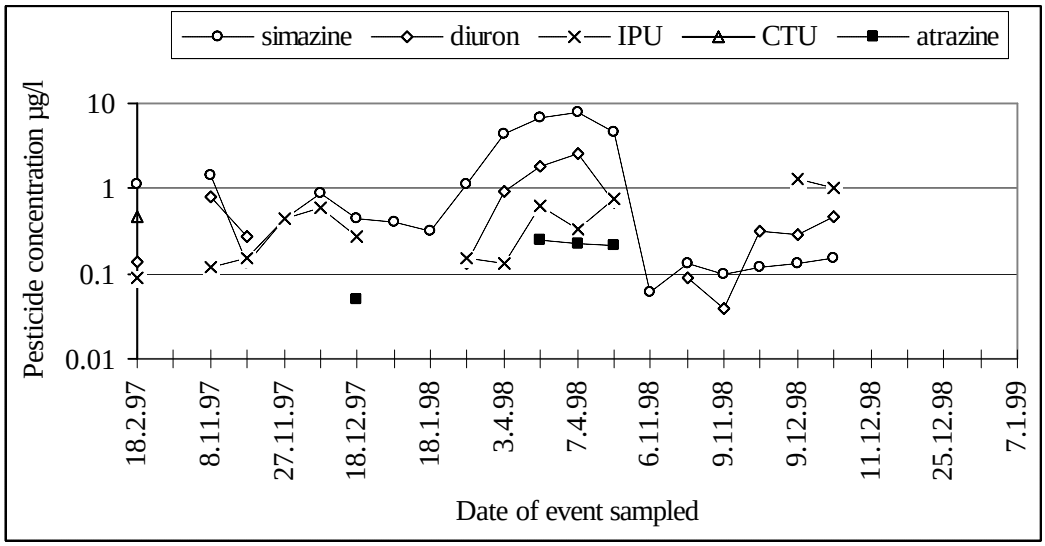

Figure 3.10 Mean concentration of pesticides (µg/l) sampled in all storm events between the 18.2.97 to 7.1.99 in the stream draining the Site C catchment. For clarity a broken line has been used to link sample dates although in many cases these represent separate storm events.

Figure 3.10 shows the mean pesticide concentrations measured in the stream at Site C following storm events which occurred between February 1997 and January 1999. The results of previous studies indicate that pesticide concentrations of toxicological significance generally occur during autumn and winter periods when soils are saturated, therefore summer events were not sampled for chemical analysis.

Peak concentrations of pesticides were sampled during April 1998. A representative storm event for site C during this period is shown in Figure 3.11. The concentration of simazine measured during the storm event in April 1998 was >10µg/l during peak flows. Diuron was detected at the next highest concentration, falling between 1 -10µg/l. The other two pesticides detected, isoproturon and atrazine were both below 1 µg/l for the duration of the storm event.

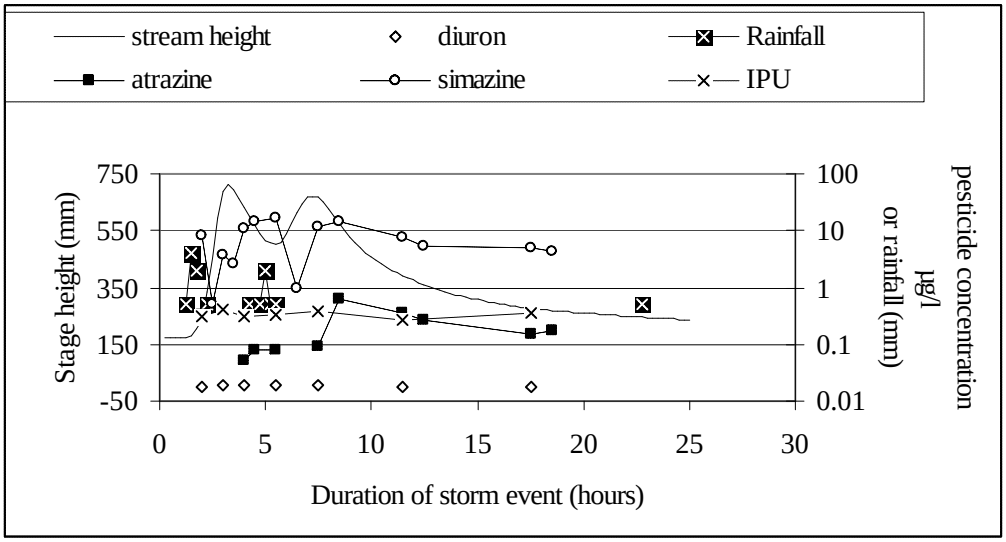

Figure 3.11 Concentration of pesticides (µg/l) sampled on the 7.4.98 in the stream draining the Site C catchment.

A range of bioassay procedures including both *in situ* and laboratory based methods were applied to samples collected during storm events at Site C. Table 3.7 shows the procedures applied to samples taken on specific dates.

Table 3.7 Bioassay procedures conducted for samples collected between winter 1998 and spring 1999 at site C.

Sample date	Storm event	Daphnia bioassay	Algal bioassay raw water either background or from event	*Kick sample for Invertebrates	Gammarus in situ mortality	sample for AChE raw water	sample for SPE
11.11.97	√	√	√			√	√
20.11.97				√			
27.11..97-14.1.98	√	√	√	√(11.12.98) √(14.1.98)		√	√
21.1.98	√	√	√			√	
27.1.98 -25.2.98			√	√(11.2.98)	√(25.2.98)	√	
4.3.98	√	√	√		√	√	√
11.3.98			√	√	√	√	
17.3.98			√		√	√	
25.3.98					√	√	
2.4.98			√		√	√	
7.4.98					√	√	
15.4.98	√	√	√	√	√	√	√
22.4.98	√	√	√		√	√	√
29.4.98	√	√	√		√	√	√
6..5.98 -10.6.98			√	√(13.5.98) √(10.6.98)	√	√	
10.6.98				√	√	√	
17.6.98	√		√		√	√	√
23.6.98 -14.10.98				√(7.7.98) √ (5.8.98) √(7..9.98) √(2.10.98)	√	√	
21.10..98	√		√		√	√	√
29.10..98					√	√	
4.11.98	√				√	√	√
11.11..98	√				√	√	
18.11.98-2.12.98				√ (2.12.98)	√	√	
9.12.98	√				√	√	√
16.12.98- 21.12.98					√	√	
30.12.98-27.1.99	√			√(6.1.98)	√	√	√
3.2.99-2.3.99				√(3.2..99) √(2.3.99)	√	√	
9.3.99	√				√	√	√
17.3.99-21.4.99				√(29.3.99)	√	√	
27.4.99	√			√	√	√	√
5.5.99-25.5.99				√(25.5.99)	√	√	√
3.6.99	√				√	√	√
9.6.99	√				√	√	√
15.6.99					√	√	√

*Drift samples were collected on each visit

3.3.2 Direct toxicity testing

Mortality of only 5 to10% were recorded in direct toxicity tests of water from automatically sampled events at Site C, using the crustacean *Daphnia magna.*

Figure 3.12 shows the mortality of *Gammarus pulex* maintained *in situ* at site C. Mortality bears some relationship to increased stream flow following rainfall. Between storm events *Gammarus* mortality was between 0 to 13%. However following several storm events mortality increased reaching a maximum value of 40% following a storm event in January 1999.

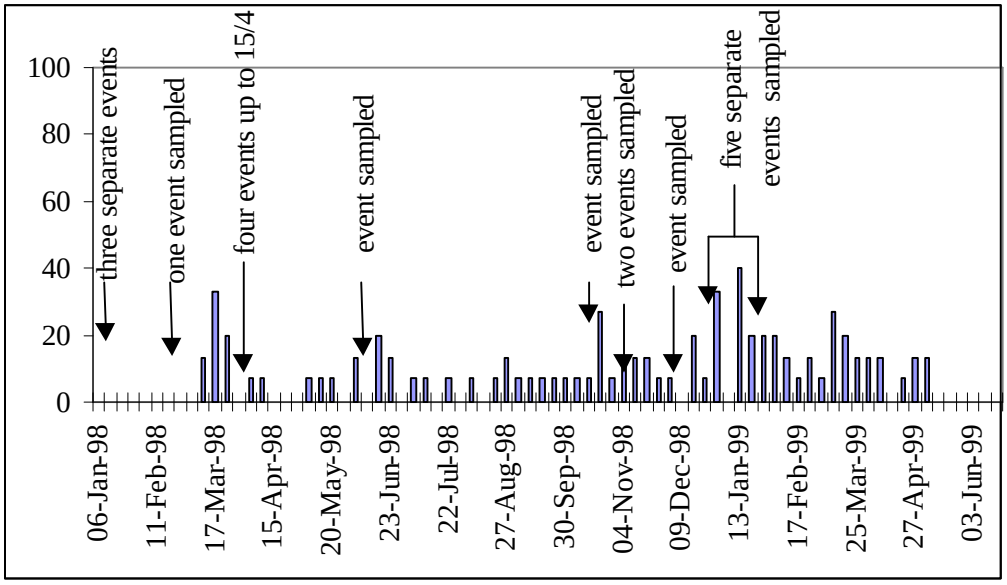

Figure 3.12. Percentage mortality of *G.pulex* deployed at site C between spring 1998 and summer 1999. The dates when the automatic sampler was triggered during increased stream flow are shown.

None of the pesticides known to be applied before the first high mortality of *Gammarus,* during March 1998, were of high toxicity to crustaceans e.g. simazine, diuron, dithianon, copper oxychloride or metalaxyl, and only some of these compounds were detected in samples in any case. Prior to the high mortality of *Gammarus* in autumn 1998 there had been a full series of pesticide applications during the summer which included the insecticides clofentezine, tebufenpyrad and chlorpyrifos. All of these chemicals would be defined as improbable leachers when their physico-chemical characteristics are considered (Gustafson , 1989). Although unlikely to leach, chlorpyrifos has been measured at toxic concentrations in a headwater stream following a storm event (Matthiessen et. al., 1995). Chlorpyrifos was not however detected in these samples. The other two pesticides were not analysed for and if present are more likely to have been associated with organic or soil particles, although these still might be bioavailable. The increased mortality in *Gammarus* can therefore not be clearly related to the presence of specific compounds.

In addition to running animal bioassays, the unicellular green alga *Raphidocellis subcapitata* was grown in samples collected from each site during periods of increased flow. Samples collected from site C (Figure 3.13) show some evidence of nutrient limitation in late spring and early summer, the samples without nutrient addition showing poorer growth. There are two occasions on which growth was relatively low: mid-January and mid-June. Since nutrient enrichment in these samples did not produce growth enhancement above that of the un-spiked samples, and growth was generally low it is possible that this is evidence of phytotoxicity. Measured herbicide concentrations were highest during April 1998 and algal growth in these samples was not apparently reduced. This suggests that reduced growth in the January and June samples may be due to unmeasured compounds or to the absence of an essential nutrient or vitamin which was not provided by the nutrient spike.

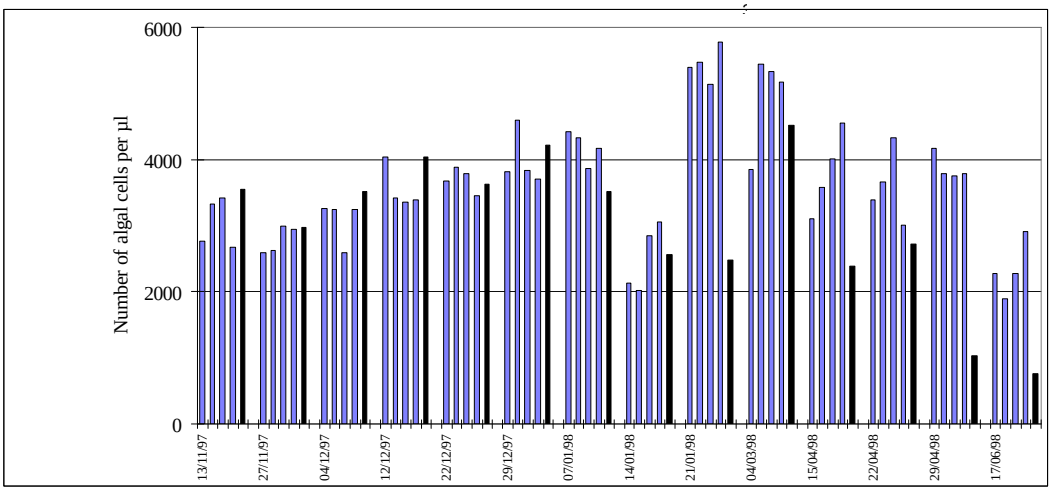

Figure 3.13. Growth of *Raphidocellis subcapitata* in bulk water samples collected from the stream at site C during periods of increased flow. The black bars denote samples from a batch that did not have extra nutrients added.

3.3.3 *Gammarus pulex* in situ feeding rate bioassay

Feeding rates of the amphipod crustacean *Gammarus pulex* were measured for site C and associated control between the beginning of March and the end of May 1997. Figure 3.14 shows feeding rates measured at site C and its associated control. The initial period in the field is characterised by a low feeding rate. This low result was probably a result of technique related stress and mortality which was subsequently reduced by modification in the deployment procedure. In the latter half of the study feeding rates increased and were comparable between the main study site and the control.

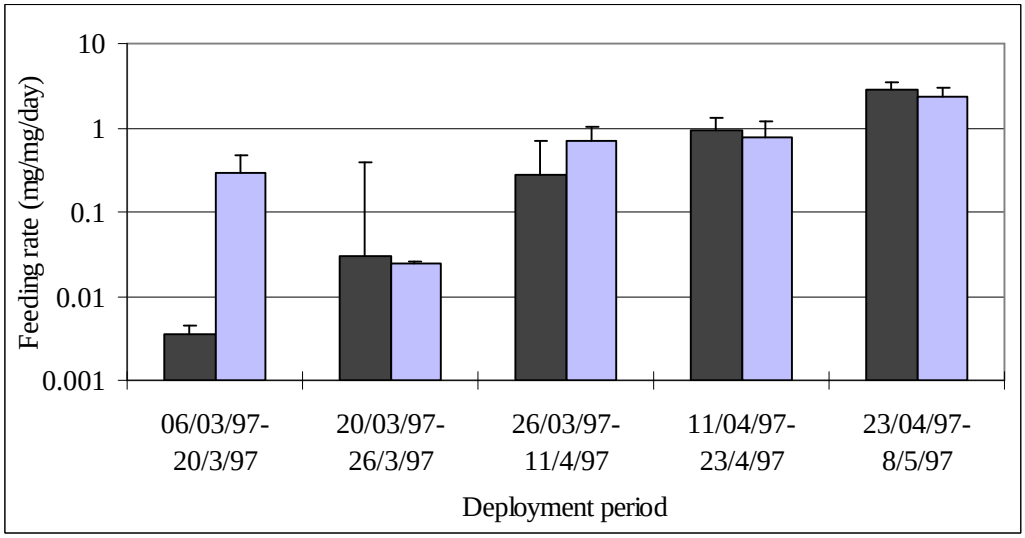

Figure 3.14. Mean (and 95% confidence limits) feeding rate of *Gammarus pulex* measured at Site C (Harpers Farm, dark bars) and associated control site (light bars)

3.3.4 Acetycholinesterase (AChE) inhibition bioassay using *Gammarus pulex*.

Site C (Harpers Farm) was initially chosen for AChE monitoring as the use of chlorpyrifos, an OP pesticide, had been confirmed in that catchment.

It had been established in laboratory studies that acetylcholinesterase inhibition resulting from exposure to OP pesticides can still be measured after a 7 day period. It was therefore established that it was still practical to use this bioassay procedure for prolonged exposure periods in the field. Figure 3.15. shows acetylcholinesterase activity in *Gammarus pulex* held for 6 to 7 days at site C between November 1997 and January 1998. The organophosphorus insecticide Dursban, which is a formulation of 48% of the active substance chlorpyrifos was applied in the orchards at site C in April and June 1997. Its half-life in soil can be up to 3 months and it has been shown to produce mortality of *Gammarus* exposed to field runoff following application. The AChE bioassay procedure was used in the latter part of 1997 to determine whether this insecticide persists at sufficient concentrations in soil to be detected in field runoff following winter rainstorms. The results indicate the absence of AChE inhibiting compounds at sufficient concentrations to produce measurable effects in this bioassay procedure.

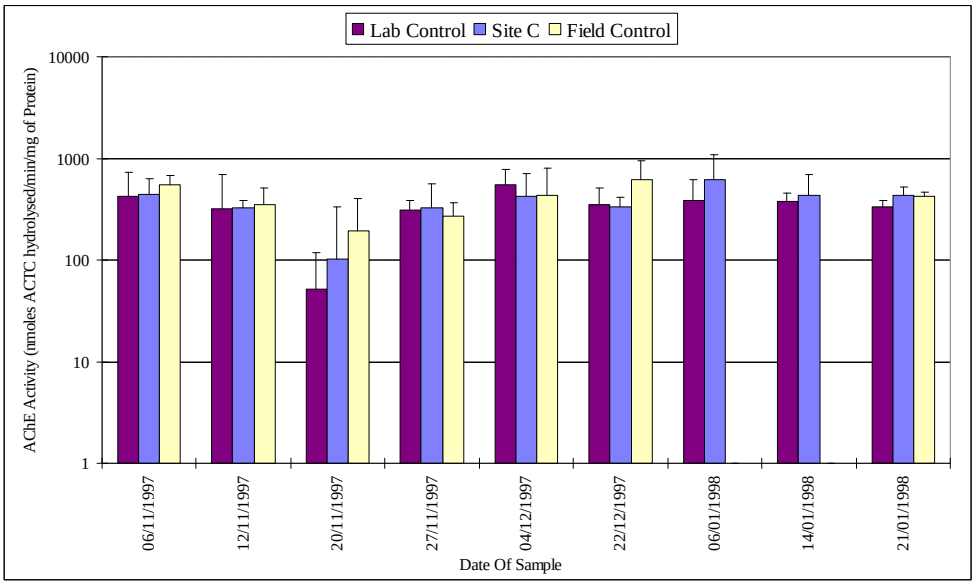

Figure 3.15. shows acetylcholinesterase activity in *Gammarus pulex* held for 6 to 7 days at site C, the Cranbrook control site and the laboratory control, between November 1997 and January 1998

Figure 3.16 shows the percentage AChE inhibition which results after 24 hours exposure to a range of chlorpyrifos concentrations. A concentration of 1.2 ug chlorpyrifos/L produced significant inhibition of AChE (47%) in *G.pulex* relative to control levels ($p<0.05$). This demonstrates that the AChE inhibition assay is highly sensitive to OPs and confirms that significant amounts of AchE inhibition could not have been present during the events described above.

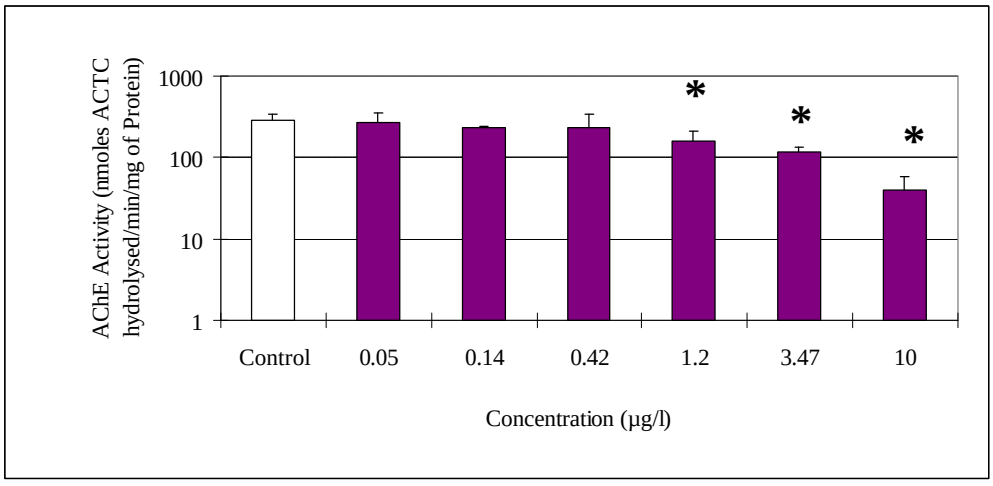

Figure 3.16. AChE activity in *Gammarus pulex* after 24 hours exposure to a range of chlorpyrifos concentrations. * (AChE levels significantly lower than control $p<0.05$)

Gammarus were also exposed to water samples collected during events at site C to determine whether cholinesterase inhibiting insecticides were present at sufficient concentration to produce measurable AChE inhibition (Figure 3.17). The AChE activity measured in animals exposed for 24 hours was comparable to that of unexposed (control) animals from the same source.

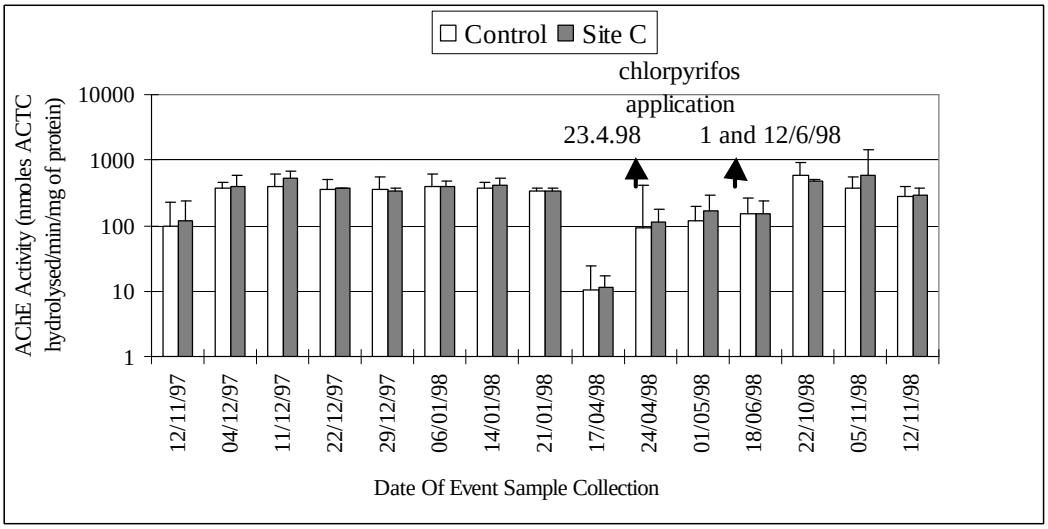

Figure 3.17 Mean (and 95% confidence limits) AChE activity In *Gammarus pulex* exposed for 24 hours to either stream water collected from site C or laboratory control water.

On two separate sampling occasions when macroinvertebrates were collected from each of six sampling stations along the Curtisden Green stream at Harpers Farm (Site C) (Figure 3.18) batches of *Gammarus pulex* were sampled for AChE analysis. Figure 3.19 and 3.20 show AChE activity in *Gammarus* from each of six sampling stations and from the control stream at Cranbrook.

Insufficient *Gammarus* were present at stations 2 and 4 during the March survey and from station 4 during June for AChE activity to be determined for these. The AChE activity at each station exhibited high inter-replicate variability. Activity was generally much lower than that observed in animals used in the cage deployment trials. In the March survey the AChE activity was higher in animals from the Cranbrook field control by comparison to that from Site C. However this difference was shown not to be statistically significant ($p>0.05$). In the second survey during the latter part of June AChE activity was once again low, with the exception that animals at station 6 had slightly elevated activity and those from the Cranbrook site were comparable to those from Site C.

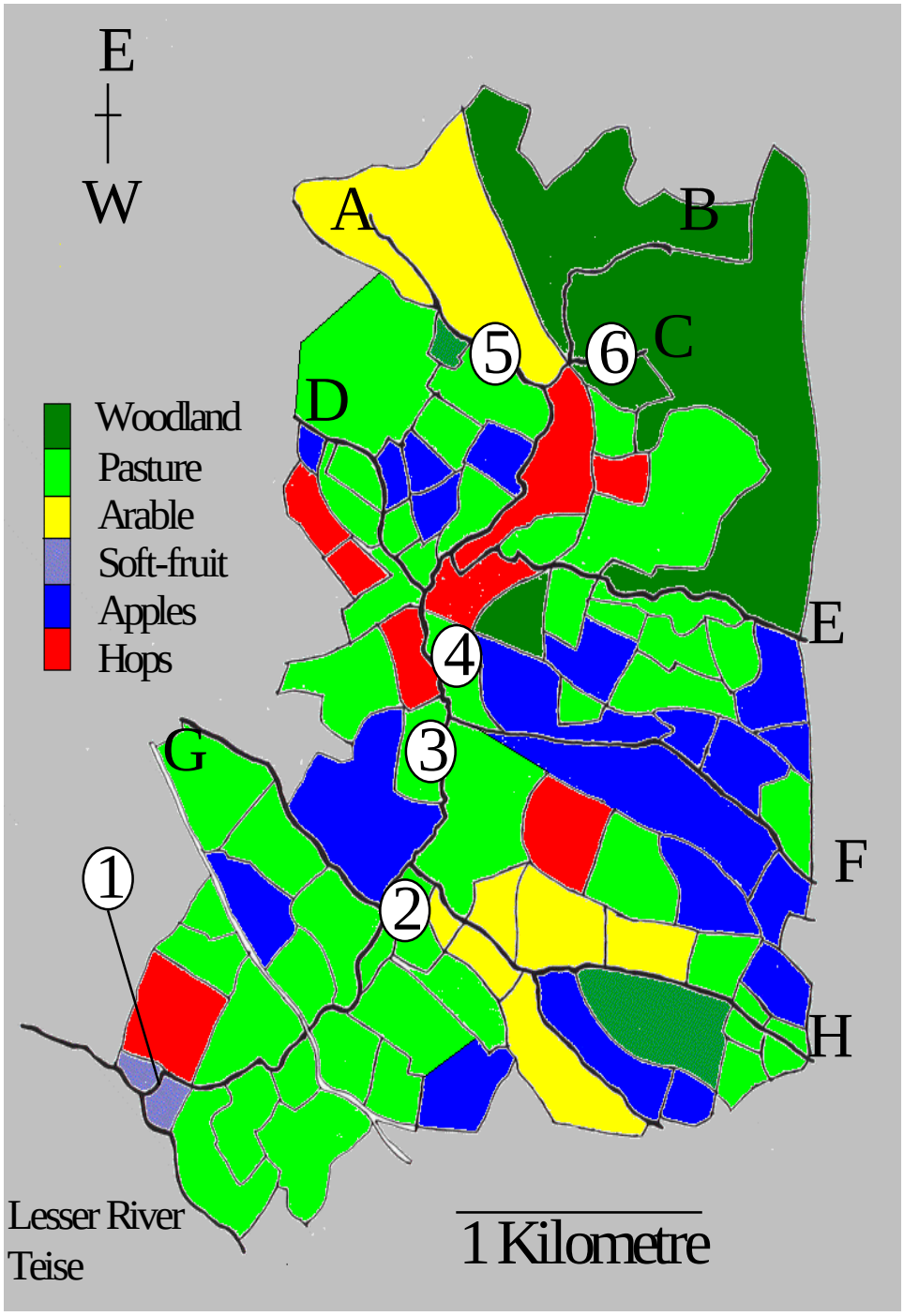

Figure 3.18 Field map of the catchment at Harpers Farm (Site C) showing crops grown during 1998 and the kick sampling stations on the Curtisden stream which were sampled on two separate occasions. A to H are tributary streams.

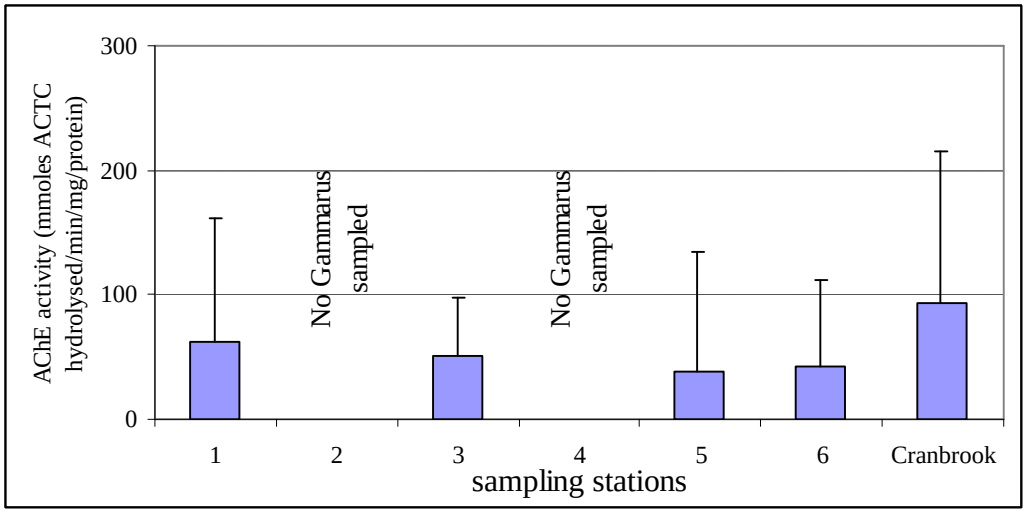

Figure 3.19 Mean (and 95% confidence limits) AChE activity in *Gammarus pulex* collected on 26.3.98 in kick samples from sites 1 to 6 at site C, or the control stream at Cranbrook

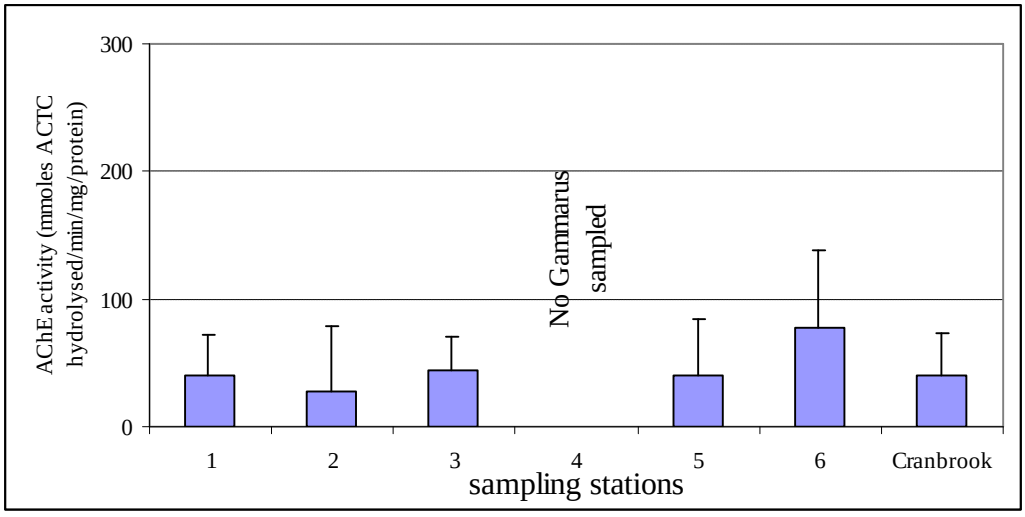

Figure 3.20 Mean (and 95% confidence limits) AChE activity in *Gammarus pulex* collected in kick samples on 24.6.98 from sites 1 to 6 at site C, or from the control stream at Cranbrook

These results are difficult to interpret given the high inter-replicate variability. It appears that AChE activity at site C was generally low but that the factors responsible for this also operate at the field control site at Cranbrook.

Very low levels of AChE activity have been measured in groups of animals collected from a range of streams other than those from the study sites and these values have been shown to increase with holding time in the laboratory. It has not been possible, however, to link these findings with information which indicates that exposure to insecticides has occurred. More importantly, the low levels of AChE activity observed in some natural populations do not

appear to be associated with detrimental effects, yet exposure to insecticides in the laboratory which have produced the same degree of inhibition often result in mortality.

3.3.5 *Chironomus riparius* sediment bioassay

Sediment samples collected from six stations at Harpers Farm (Site C) (Figure 3.18) were tested using the *Chironomus riparius* 10-day growth test. The presence of toxic compounds at biologically significant concentrations in the sediment is indicated by poor growth of the Chironomid larvae or in worst cases by mortality. Sediment samples were collected from Site C on three separate occasions. The growth of larvae in these sediment samples is shown in Figure 3.21 to 3.23.

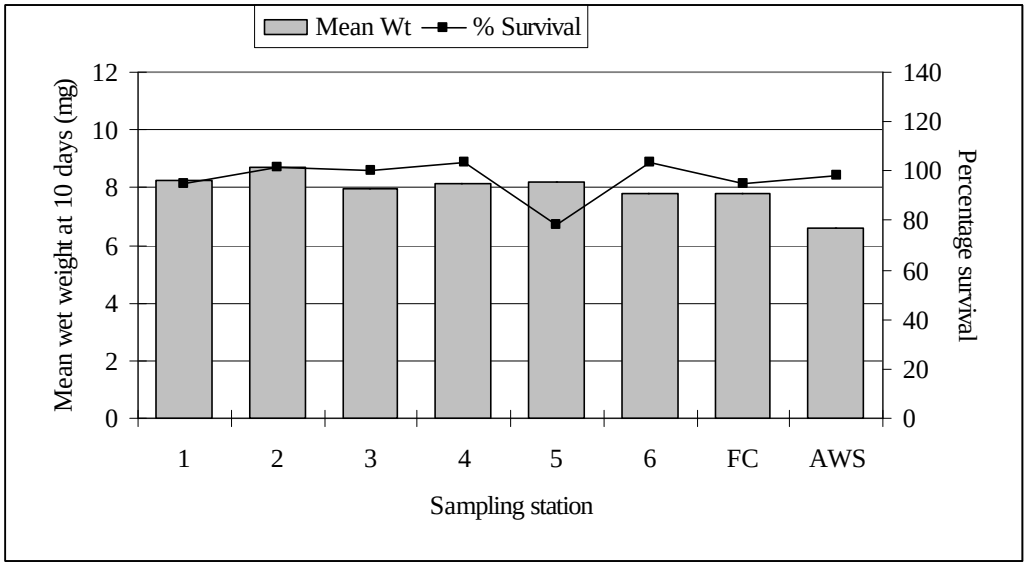

Figure 3.21 Mean (and 95% confidence limits) growth of *Chironomus riparius* in sediment samples collected on the 26.3.98 stations 1 to 6 at site C the control stream at Cranbrook or in acid-washed sand (AWS). Percentage survival of adult Chironomids is also shown.

Although all larvae were fed a comparable ration of food during the study the added nutritional content of the sediments generally resulted in higher larval growth at all stations relative to that in the acid-washed sand. Growth was highest across all stations in the sediments collected in March and June. Growth was similar to that in the acid-washed sand in the October sediments. Growth in station 5 sediment was marginally lower in June and in station 6 sediment sampled in October, growth was particularly reduced.

Stations 1, 2 and 4 are the closest to the Orchards and Hop gardens but the results indicate that relatively poorer growth occurs at stations 5 and 6 although growth in sediments from all stations collected in October is poorer.

Most of the insecticides applied to the Orchards and Hop gardens had been applied before the sediments were sampled during June. There had also been increased flow in the stream following rainfall in mid-June. Water flow had also increased, however, following rainfall the day before the October sediments were sampled.

The results of the sediment growth study indicate that there is some factor operating to reduce growth in the stations 5 and 6 in March and June respectively and in all the October samples.

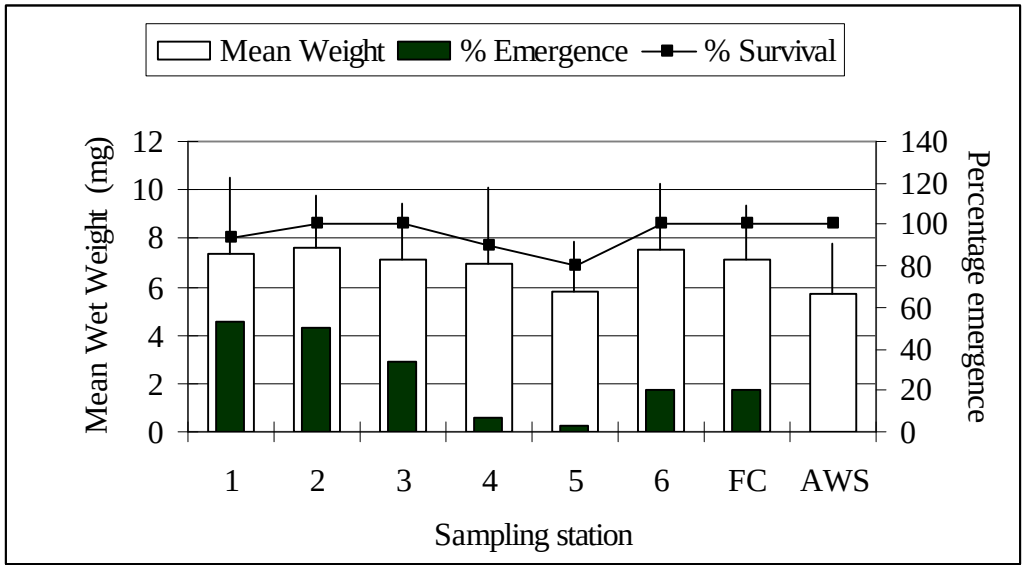

Figure 3.22 Mean (and 95% confidence limits) growth of *Chironomus riparius* in sediment samples collected on the 24.6.98 stations 1 to 6 at site C the control stream a Cranbrook or in acid-washed sand (AWS). Percentage survival and emergence of adult Chironomids is also shown.

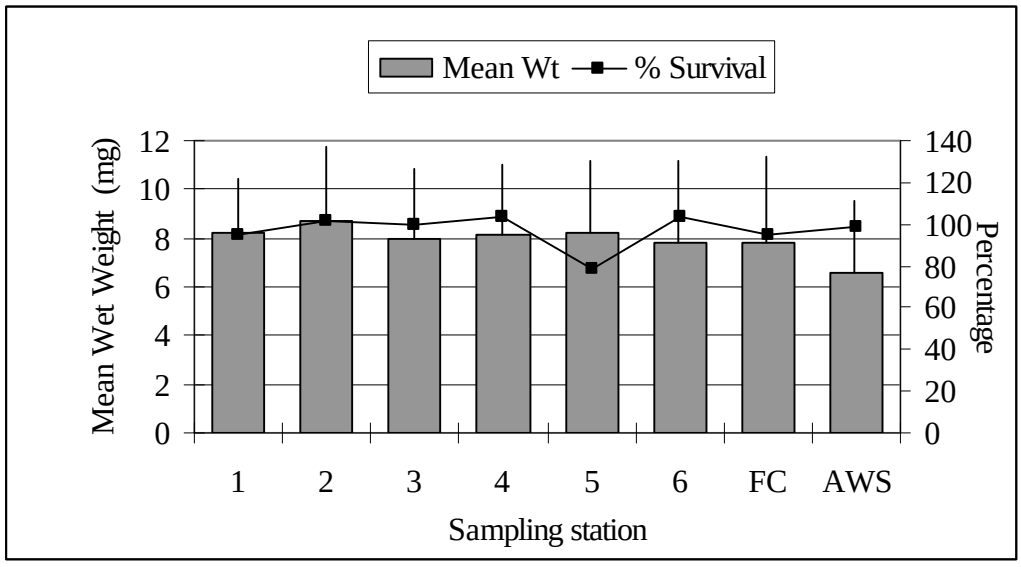

Figure 3.23 Mean (and 95% confidence limits) growth of *Chironomus riparius* in sediment samples collected on the 22.10.98 stations 1 to 6 at site C the control stream at Cranbrook or in acid-washed sand (AWS). Percentage survival of adult Chironomids is also shown.

3.3.6 Toxicity tests of Solid-phase (SPE) extracts

A selection of SPE samples collected during storm events from the stream draining Harpers Farm (Site C) were tested using a range of bioassay procedures.

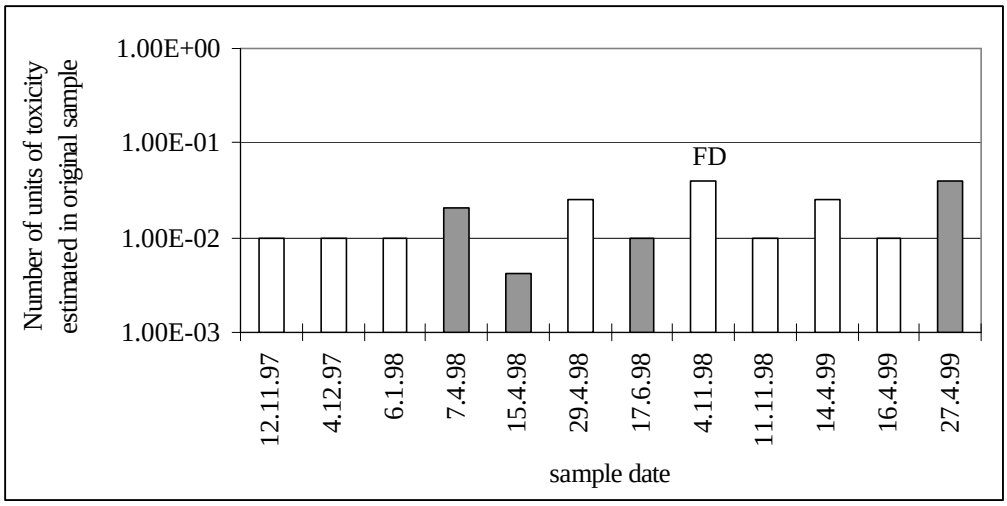

Figure 3.24 Graph showing estimated toxic units in stream or field drain (FD) samples collected from site C during storm events. Toxic units are based on the results of toxicity tests of solid-phase extracts using the Cladoceran *Daphnia magna.* The blank bars indicate the detection limit for those tests in which no effects were observed, and are derived from the SPE concentration factor.

Figure 3.24 shows the number of units of toxicity estimated to be present in solid-phase extracts from storm event samples collected at Site C. Only 3 of 12 samples from the stream showed more than 0.01 units of toxicity.

A selection of SPE samples collected during storm events from the stream draining Harpers Farm (Site C) were tested for acetylcholinesterase activity using *Chironomus riparius*.

Figure 3.25 shows relative natural activity level of 5 and 10 day old larvae. The older larvae were used for the test procedure because of their greater relative AChE levels. Figure 3.26. shows the acetylcholinesterase activity level of 10 day old chironomids exposed to SPE samples from Site C. Although there is some variability in the AChE levels of animals exposed to different samples none of the differences were shown to be significant ($p>0.05$).

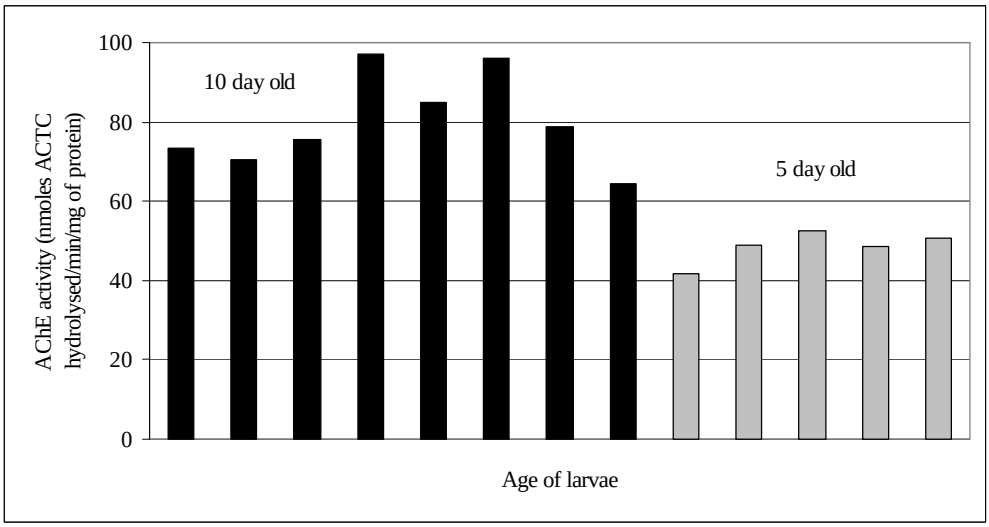

Figure 3.25 Natural AChE activity of individual *Chironomus riparius* in two age groups sampled from the laboratory culture.

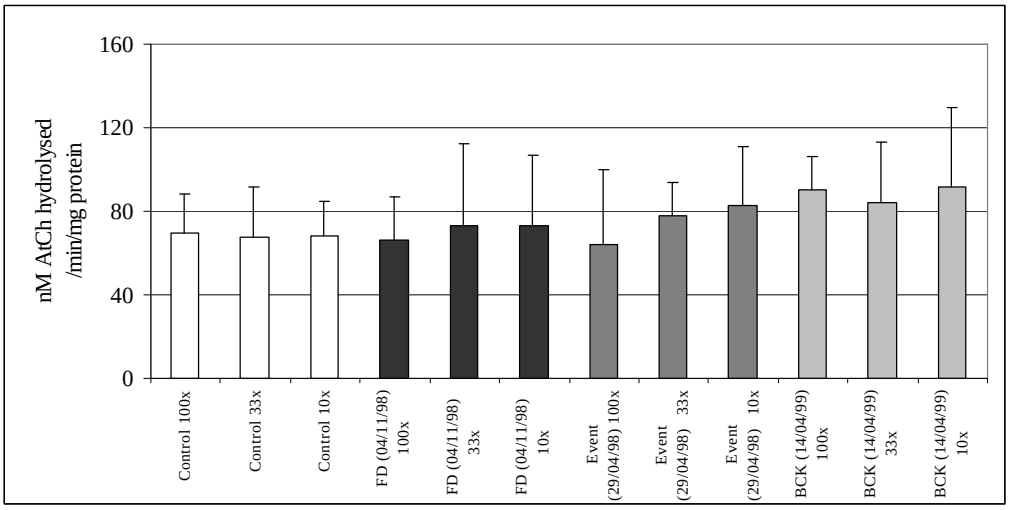

Figure 3.26 Mean AChE activity (and 95 % confidence limits) of *Chironomus riparius* exposed to concentrated base-flow (BCK), field drain (FD), and storm event samples from the stream at Harpers Farm (Site C)

For the bioassay using growth of the green alga *Raphidocellis subcapitata* as a measure of toxic effect, a unit of toxicity is defined as that concentration of one or more compounds required to produce 50% inhibition in the growth of a unicellular green algal species. Toxicity data for individual compounds were derived from the data presented in Table 3.5.

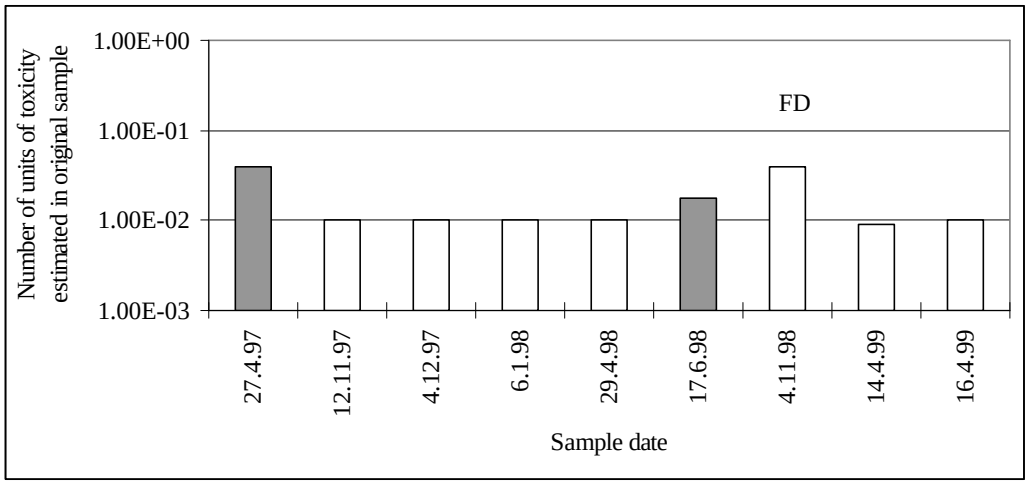

Figure 3.27 Graph showing estimated toxic units in stream or field drain (FD) samples collected from site C during storm events. Toxic units are based on the results of toxicity tests of solid-phase extracts using the green alga *Raphidocellis subcapitata.* The blank bars indicate the detection limit for those tests in which no effects were observed, and are derived from the SPE concentration factor.

Figure 3.27 shows the number of units of toxicity estimated to be present in solid-phase extracts from storm event samples collected at Site C using the green alga (*Raphidocellis subcapitatum)* bioassay. Seven of the eight samples taken from the main stream showed less than one unit of toxicity. One sample from spring 1997 did contain a higher level of toxicity, 4 toxic units were measured in the sample taken on 27.4.97.

The level of response of the macrophyte *Lemna minor* in the bioassay of SPE extracts of a number of event samples from sites C suggests the presence of approximately 1 µg/L equivalent of photosynthesis inhibiting herbicides (this is illustrated for site C in Figure 3.28). Paraquat is added to all samples, and produces a rise in conductivity. Samples, which also contain photosynthesis inhibiting herbicides, produce a reduction in conductivity as compared to that produced by paraquat alone. The magnitude of the difference between conductivity levels in the paraquat only treatment and those treatments with added SPE extracts, is proportional to the concentration of photosynthesis inhibiting herbicides present. Figure 3.28 shows the results of a *Lemna* bioassay conducted on an SPE extract of a sample collected on the 29.4.98 at Harpers Farm (site C). Although conductivity decreases with increased sample concentration factor, only at a concentration factor of 100x is the mean conductivity significantly less than that of the paraquat treatment. If it is assumed that the herbicidal activity was due to atrazine alone this would be equivalent to an atrazine concentration of approximately100µg/l in the 100x SPE sample. This would indicate a raw water concentration of 1 µg/L of atrazine or other herbicides with a similar mode of action. Although the pesticide concentration in this sample was not measured, 4.7µg/l of simazine was measured in an event sampled two weeks earlier and atrazine, isoproturon and diuron from the same event were present at concentrations <1µg/l. The herbicide concentration estimated from the assay result therefore does not appear unrealistic.

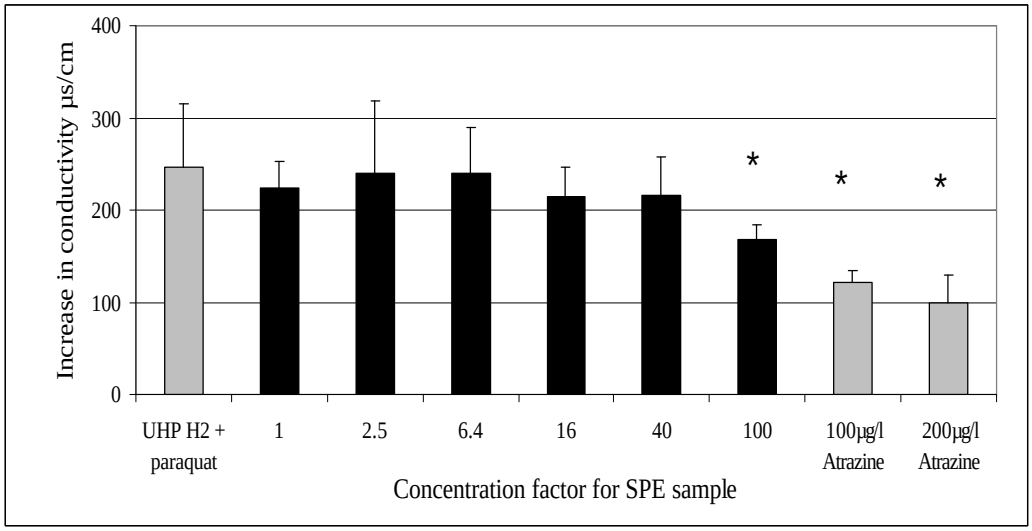

Figure 3.28 Mean (95 percent confidence intervals) increase in conductivity over 48 hours in Lemna minor bioassay of SPE extract from an event sample collected on 29.4.98 at Site C
(* Significantly different from the control p<0.05)

3.3.7 Predicted versus measured toxicity

Water samples collected at site C during periods of increased flow between spring 1997 and winter 1998 show very low potential toxicity to *Daphnia magna*, based on the compounds detected in the chemical analysis. However the actual toxicity of water sample extracts on two of the sampling occasions was much higher than predicted (Figure 3.23). Water sample SPE extracts collected on 7.4.98 and 15.4.98 gave lethal responses at concentration factors of 2.5 to 100 using *Daphnia magna*. The results indicate that the majority of toxicity was due to compounds other than those specifically analysed for and detected in samples. For these samples the remainder of the extract was fractionated by semi-preparative high performance liquid chromatography (HPLC). Re-testing using the Daphnia bioassay identified individual toxic fractions.

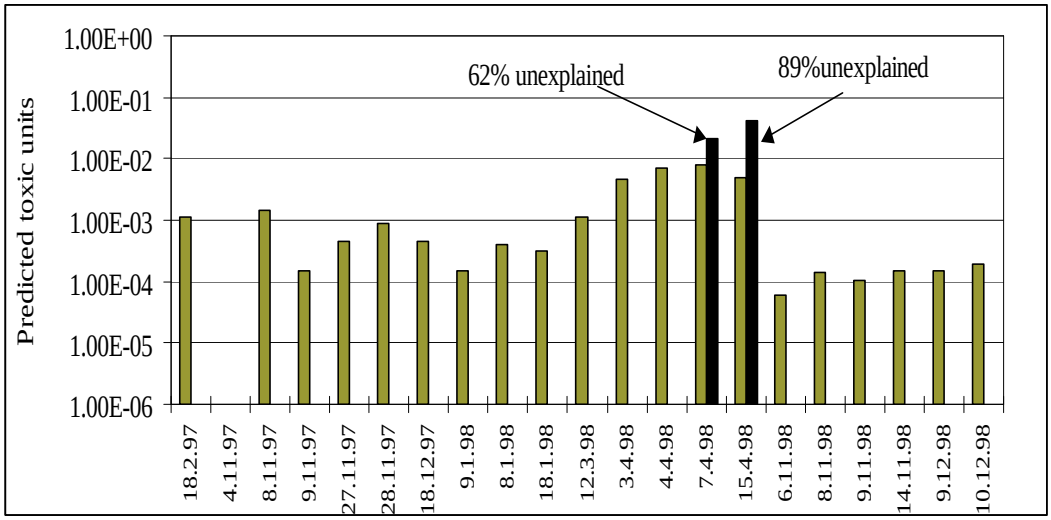

Figure 3.29 Graph showing predicted toxic units* for the Cladoceran *Daphnia magna* based on the compounds detected in water samples collected during increased stream-flow, following rainfall at site C. The dark bars show the number of toxic units calculated on the basis of a Daphnia bioassay of the SPE extracts of stream samples.

***To calculate the toxic units, the concentration of each compound measured in a water sample is divided by the relevant toxicity figure for Daphnia magna from Table 3.5 and these values are then summed.**

Individual toxic HPLC fractions were analysed by Gas Chromatography - Mass Spectrometry (GC-MS) in an attempt to identify individual toxic compounds. In the sample from 7.4.98 no other toxic compounds were positively identified. However for the sample extract from 15.4.98 three compounds, the insecticide endosulfan sulphate, the herbicide pendimethalin and the nonionic surfactant degradation product nonylphenol were positively identified as present.

Predicted toxicity of water samples collected during storm events at Site C is shown in Figure 3.30. The predicted toxicity of samples from this site to algae was higher than that for *Daphnia magna*. This is because it was predominantly herbicides that are less toxic to invertebrates that were present in the samples. The measured toxicity in samples from November and December 1997 was less than 1 unit of toxicity this is in broad agreement with the predicted figures.

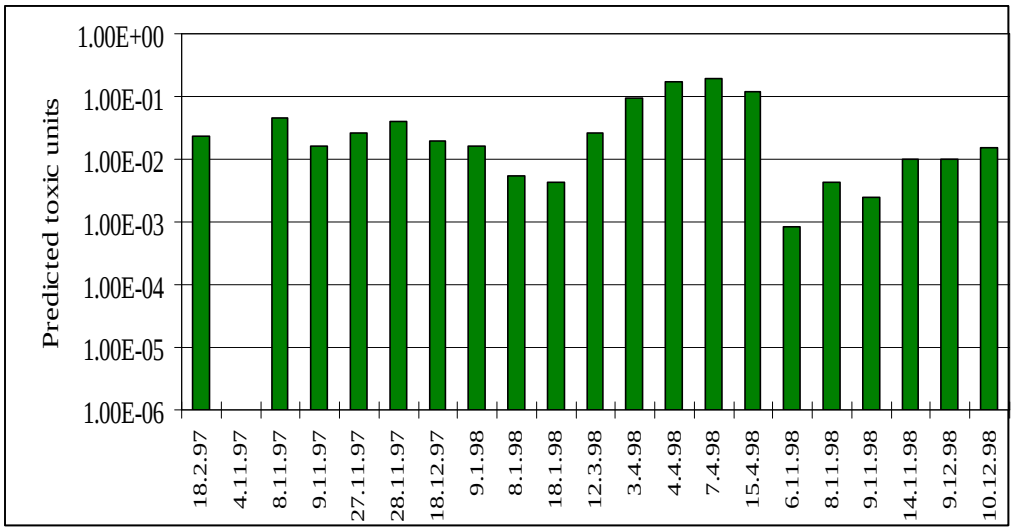

Figure 3.30 Graph showing predicted toxic units* for the green alga *Raphidocellis subcapitata* based on the compounds detected in water samples collected during increased stream-flow, following rainfall at site C.

***To calculate the toxic units, the concentration of each compound measured in a water sample is divided by the relevant toxicity figure for Raphidocellis, from Table 3.5 and these values are then summed.**

3.3.8 Sampling of indigenous macroinvertebrates, in drift nets or kick samples

Figure 3.31. Shows the Shannon diversity index for drift and in kick samples taken at Harpers Farm (site C) over a period in which several separate runoff events occurred. The Shannon diversity index is more commonly calculated for in situ communities of organisms. In this case when applied to drift samples it provides a means of comparing the diversity of drifting organisms to that of organisms present in the stream bed. Figure 3.31 also shows the total number of organisms in the drift. If diversity scores of drift samples and kick samples are comparable during the same sampling period this may indicate either a large increase in flow or increased drift of organisms due to the presence of toxic compounds in the stream water. Since some species are more likely to be found in the drift than others, high species diversity in drift samples collected during storm events may also indicate the presence of toxicity in the water column.

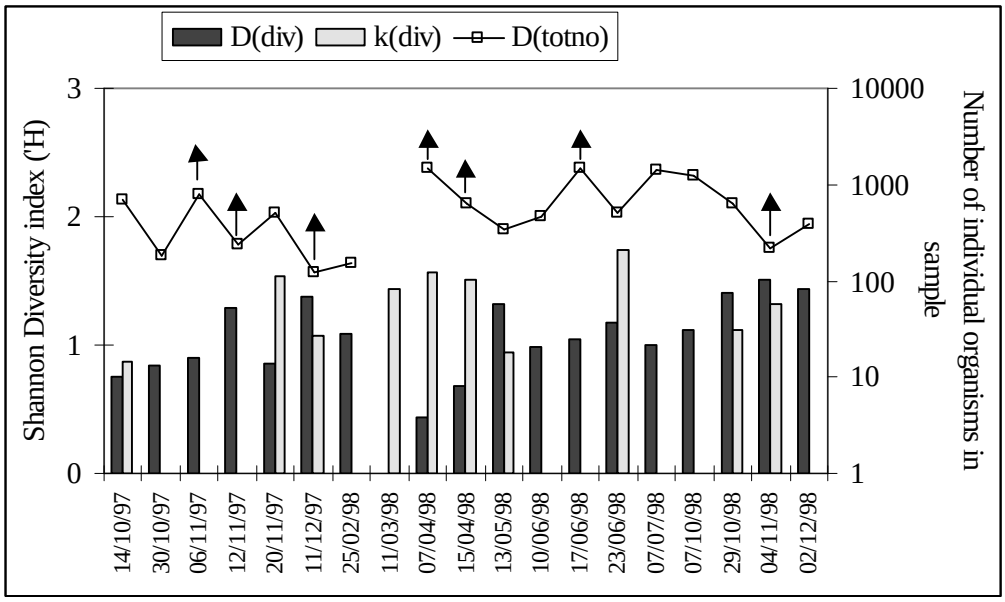

Figure 3.31. Shannon diversity index for macroinvertebrates sampled in drift nets (dark bars) or kick samples (light bars) collected at Harpers Farm (Site C). The broken line indicates total numbers of organisms in the drift samples. Arrows indicate that a runoff event occurred during the week proceeding the date on which a sample was collected.

The dominant groups in the drift at site C were chironomids, oligochaetes and Asellus. During the autumn in 1998 *Gammarus pulex* was also observed in moderate numbers in the drift, and its presence was also noted in kick samples during the same period. Samples taken at the control for site C (Cranbrook) showed a greater diversity. Two different species of mayfly nymph, three species of stonefly nymph and two species of caddis larvae were also sampled during 1997 and 1998 at this site.

3.3.9 Detailed survey of indigenous macroinvertebrates sampled by drift net and kick sampling

In addition to the regular sampling at field sites A, C and D a more detailed survey of macroinvertebrates in kick samples was conducted at Harpers Farm (site C)(Figure 3.18). Figure 3.32 shows the mean Shannon Species diversity index for each of six sampling stations on the stream which drains the catchment at Harpers Farm.
sites 1 to 4. The position of site 6, in a wooded area may have influenced this decrease in diversity. Reduction of light reaching the stream, due to increased shading by new foliage may have reduced the allochthonus food supply for stream dwelling macroinvertebrates.

Species diversity between sampling occasions appears comparable with a trend towards higher diversity at the two stations closest to the spring-fed source (5 and 6). The low diversity at sites 1 to 4 may be associated with the fact that all of these sites are close to either orchards or hopyards. On the second sampling occasion during June the sample from station 6 shows a reduced score which is more comparable to that of

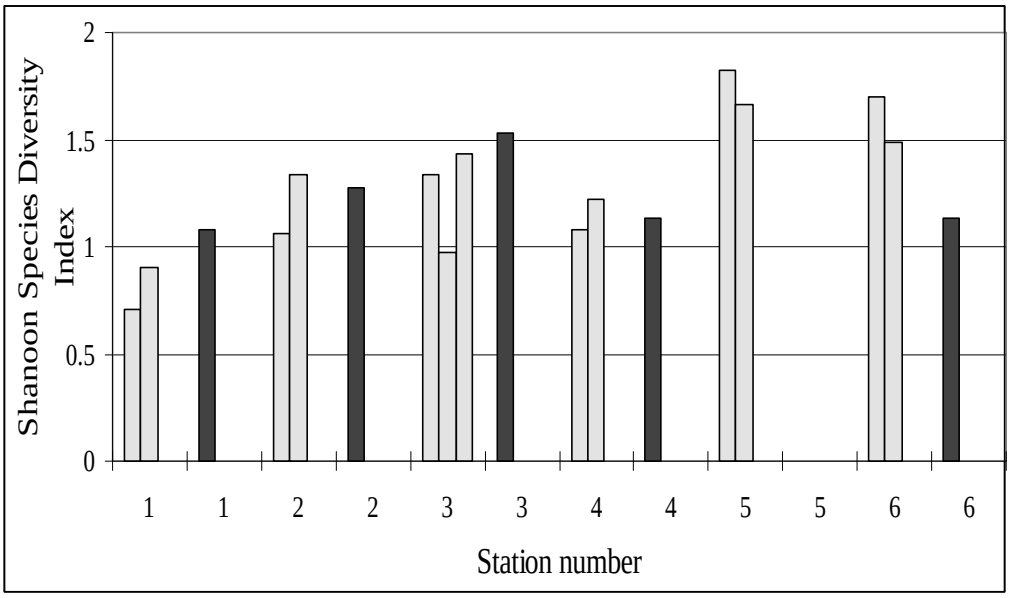

Figure 3.32. Shannon Species Diversity Index calculated for Kick samples of macroinvertebrates collected from different stations on the Curtisden Green Stream which drains the Harpers Farm catchment. The light-bars represent individual replicate samples from 26.3.98 and the dark-bars, samples collected on the 24.6.98.

3.4 Site D
3.4.1 Application data and storm event data

Table 3.8 Pesticide usage data for the study catchment D arable, (maize, linseed and wheat).
(1) compounds followed by '(a)' analysed for in samples, and those followed by '(d)' detected in samples

Sample site	Application date		Pesticide type	Active substance(1)
(D)Dollyman's Farm near Wickford	1997	March	herbicide	**isoproturon(a)**
			herbicide	**mecoprop(d)**
			fungicide	**propiconazole(a)**
			fungicide	chlorothalonil
			fungicide	flutriafol
		April	herbicide	**atrazine(d)**
			insecticide	**lindane(a)**
		May	herbicide	metsulfuron
		September	herbicide	diquat
		September	herbicide	glyphosate
	1998	January	herbicide	cycloxydim
			herbicide	**diflufenican(a)**
			herbicide	**isoproturon(d)**
			insecticide	**cypermethrin(a)**
		February	herbicide	clopyralid
			herbicide	**cyanazine(a)**
		March	growth regulator	chlormequat
		May	herbicide	**fluroxpyr(a)**
			herbicide	metsulfuron methyl
			insecticide	**cypermethrin(a)**
		June	fungicide	epoxiconazole
		August	herbicide	glyphosate
		November	herbicide	triallate
	1999	February	herbicide	diflufenican
			herbicide	isoproturon
			insecticide	cypermethrin
		March	growth regulator	chlormequat
		April	herbicide	metsulfuron methyl
			herbicide	ioxynil
			herbicide	bromoxynil

Figure 3.33. Shows a characteristic hydrograph for one of the small headwater streams (at Dollymans'Farm), peak flow is reached relatively rapidly and the whole event lasts only a few hours. Nine pesticides were measured in the water samples collected on this date, of these two were fungicides and the remainder herbicides. Figure 3.34 shows the pesticide concentrations measured at Site D between 6.5.97 and 11.11.98. In these samples the herbicides isoproturon and chlorotoluron were also measured in addition to the pesticides previously detected.

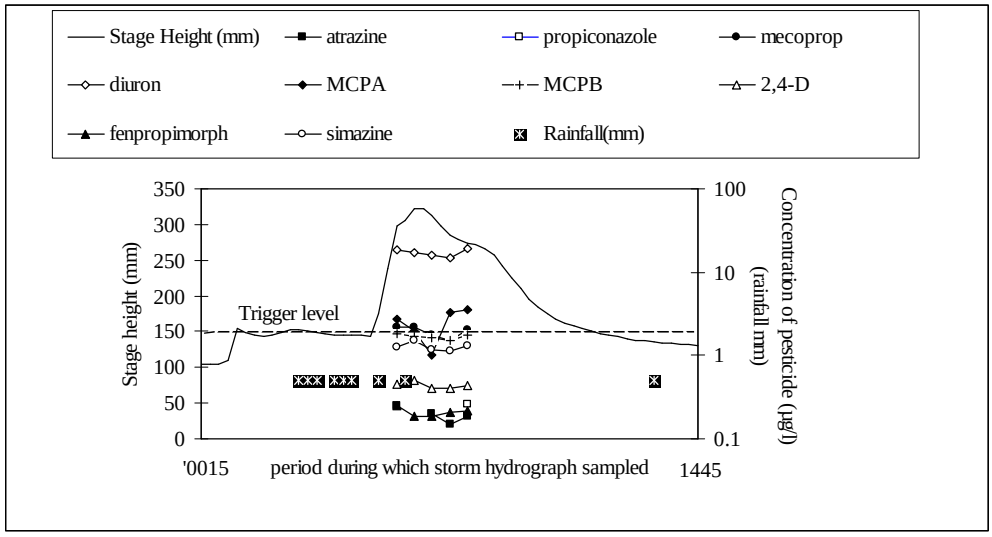

Figure 3.33 Pesticide concentrations (µg/l) measured in the stream draining the Dollymans Farm site during a storm event (6.5.97). The event was recorded between 0015 and 1445.

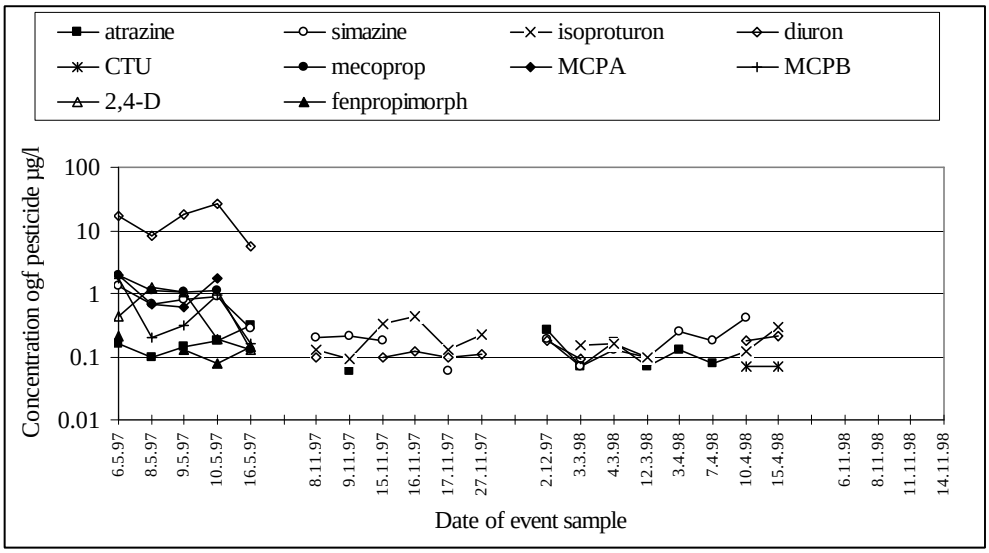

Figure 3.34 Pesticide concentrations (µg/l) measured in the stream draining the Dollymans Farm site during storm events that occurred from 6.5.97 to 11.11.98.

A range of bioassay procedures including both in situ and laboratory-based methods were applied to samples collected during storm events at Site C. Table 3.9 shows the procedures applied to samples taken on specific dates.

Table 3.9 Bioassay procedures and samples collected at Site D

Sample date	Storm event	Daphnia bioassay	Algal bioassay raw water either background or from event	*Kick sample for Invertebrates	Gammarus in situ mortality	sample for AChE raw water	sample for SPE
10.11.97	√	√	√			√	√
19.11.97	√			√			√
3.12.97	√						√
7.1.98	√	√	√		√		√
15.1.98 -1.4.98			√	√(15.1.98) (11.2.98) (11.3.98)	√	√	
7.4.98	√	√	√		√	√	√
8.4.98	√	√	√				
16.4.98	√	√	√	√	√	√	√
23.4.98	√	√			√	√	√
30.4.98-20.5.98			√		√		
28.5.98	√			√(13.5.98)	√	√	
3.6.98-7.10.98				√(5.8.98) (7.10.98)	√		
14.10.98	√	√	√				√
21.10.98	√	√	√				√
29.10.98	√	√	√				√
4.11.98	√	√	√	√			√
11.11.98							
18.11.98	√						
25.11.98-3.12.98			√				
10.12.98-16.12.98	√						
21.12.98-27.1.99				√(6.1.98)			√
3.2.99	√		√				√
10.2.99-24.2.99				√(11.2.98)			
2.3.99-9.3.99	√		√				√
17.3.99-25.3.99				√(2.3.99)			
29.3.99	√		√	√			√
8.4.99							
14.4.99	√		√				√
21.4.99							
27.4.99	√		√	√			√
5.5.99							
11.5.99	√		√				√
18.5.99-25.5.99				√(25.5.99)			
3.6.99	√		√				√

*Drift samples were collected on each visit

3.4.2 Direct toxicity testing

Bioassays of stream water samples collected from automatic samplers during increased flow at each of the sites produced some mortality in the *Daphnia magna* bioassay. At site D 100% mortality occurred in one batch of hourly samples (12 to 17 hours) sampled during an event (10.11.97). On one other occasion 25% mortality was recorded in one batch of hourly samples (13 to 18 hours) during an event (19.11.97). One field-drain sample (7.10.98) also produced 100% mortality, although no mortality was recorded in stream samples up and downstream of the drain.

Gammarus pulex deployed in cages at Site D exhibited abnormal behaviour during a period in which the pyrethroid insecticide cypermethrin was being sprayed in adjacent fields and mortality increased to 40, 73 and 53% in the three weeks following spraying.

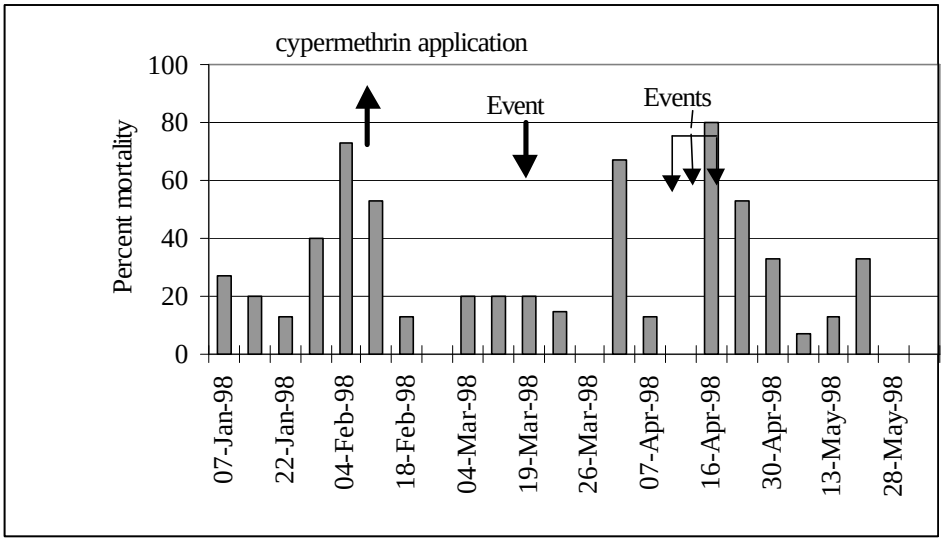

Figure 3.35 Percentage mortality of *G.pulex* deployed at site D between spring 1998 and summer in 1999. The dates when the automatic sampler was triggered during increased stream flow and the period during which the synthetic pyrethroid cypermethrin was applied in the catchment are also indicated.

Growth of *Raphidocellis subcapitata* in stream water samples collected by the automatic sampler at site D is shown in Figure 3.36. Growth appears to be nutrient limited in events sampled on the 8, 16 and 23 of April 1998. The low growth of algae in event samples from 13 and 20 of November 1997, which had nutrient additions, however, indicates that phytoactive compounds were also exerting some effects. This is also the case for the event sampled on the 28 of May 1998. Only relatively low concentrations of several herbicides were measured at this site during autumn 1997. Other unmeasured compounds, however, could also have been influencing growth of algae.

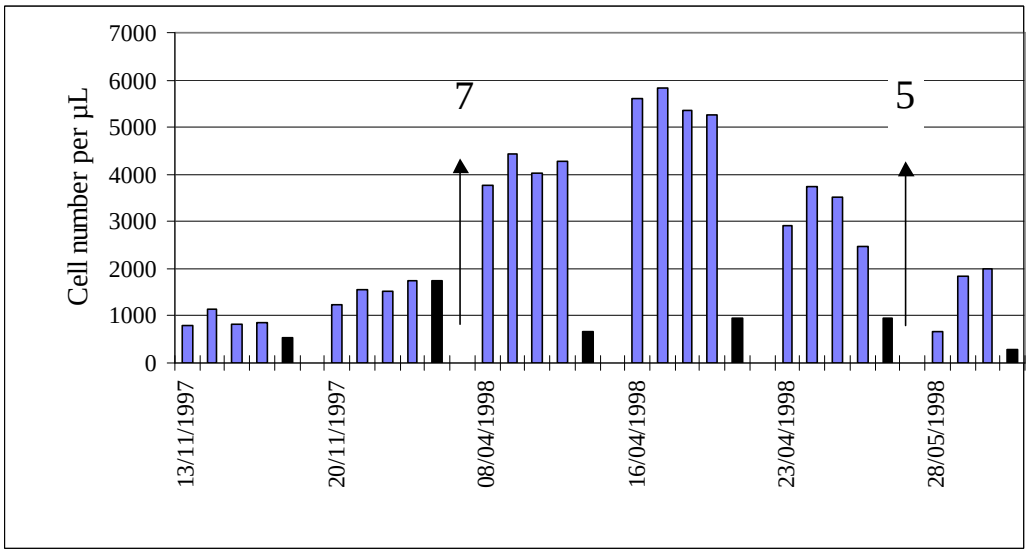

Figure 3.36. Growth of *Raphidocellis subcapitata* in bulk water samples collected from the stream at site D, during periods of increased flow. The black bars denote samples from a batch that did not have extra nutrients added. The cumulative number of pesticide applications occurring between successive samples are indicated by an arrow.

3.4.3 *Gammarus pulex* in situ feeding rate bioassay

Feeding rates of the amphipod crustacean *Gammarus pulex* were measured for sites D and C and associated controls between the beginning of March and the end of May 1997. Figure 3.37 shows feeding rates measured at site C and its associated control. The initial period in the field is characterised by a low feeding rate. This low result was probably a result of technique-related stress and mortality that was subsequently reduced by modification in the deployment procedure. In the latter half of the study, feeding rate increased and was comparable between the main study site and the control.

At site D (Figure 3.37), low initial feeding rate was also due to similar problems to those described for Site C. A series of events was also sampled during the latter part of the deployment period at this site. The first event was collected by the autosampler 26 of April 1997 after 4 mm of rainfall. Five subsequent events were sampled automatically on the 6, 8, 9, 10 and 16 of May. During the period of deployment 1.5.97 to 9.5.97 100% mortality of test animals occurred in the main site at Dollymans Farm. During the same period only 7% mortality was recorded for animals placed in the control stream at the site. At both the main site and control, caged *Gammarus* would have been exposed to a similar increase in stream flow and suspended particulates following rainfall.

The insecticide lindane was applied to fields adjacent to the stream in which the *Gammarus* were placed at the end of April 1997. Although a range of compounds were measured in the five events in early May during the period in which high *Gammarus* mortality occurred, lindane (the potentially most toxic compound) was not measured above a detection limit of 0.03 µg/L. Samples from the event on the 26.4.97, however, were not analysed for pesticides, only tested for toxicity. There is therefore a possibility that lindane contamination of the stream occurred as a result of spray-drift.

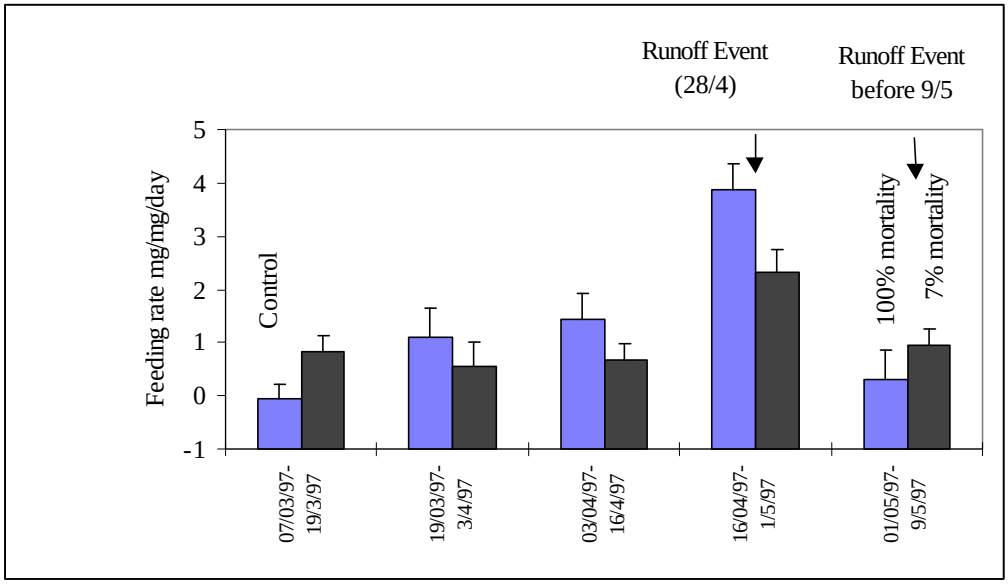

Figure 3.37. Mean (and 95% confidence limits) feeding rate of *Gammarus pulex* measured at the site D (Dollymans Farm, dark bars) and associated control site (light bars)

3.4.4 Toxicity tests of solid-phase extracts (SPE)

Toxicity tests of solid-phase extracts of water samples collected from Site D were conducted using the crustacean *Daphnia magna*, Figure 3.38.

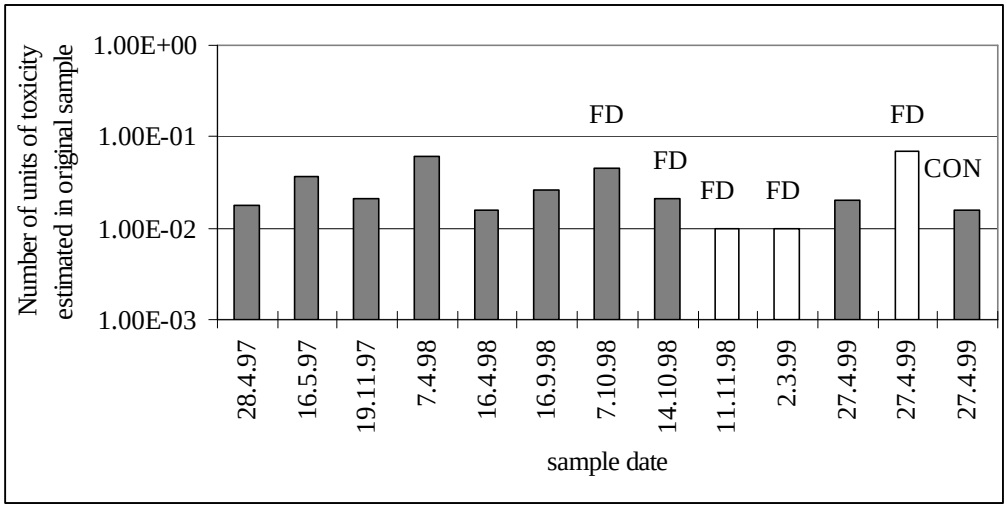

Figure 3.38 Graph showing estimated toxic units in stream or field drain (FD) samples collected from site D during storm events. Toxic units are based on the results of toxicity tests of solid-phase extracts using the Cladoceran *Daphnia magna.* The blank bars indicate the detection limit for those tests in which no effects were observed, and are derived from the SPE concentration factor.

In addition to testing storm event samples collected from the stream, samples were also collected from a field drain discharging several metres upstream of the main sampling point. The number of units of toxicity calculated for each sample based on a 100 times concentration

is shown in Figure 3.38. Ten of the thirteen samples tested showed over 1 unit of toxicity at 100 times concentration.

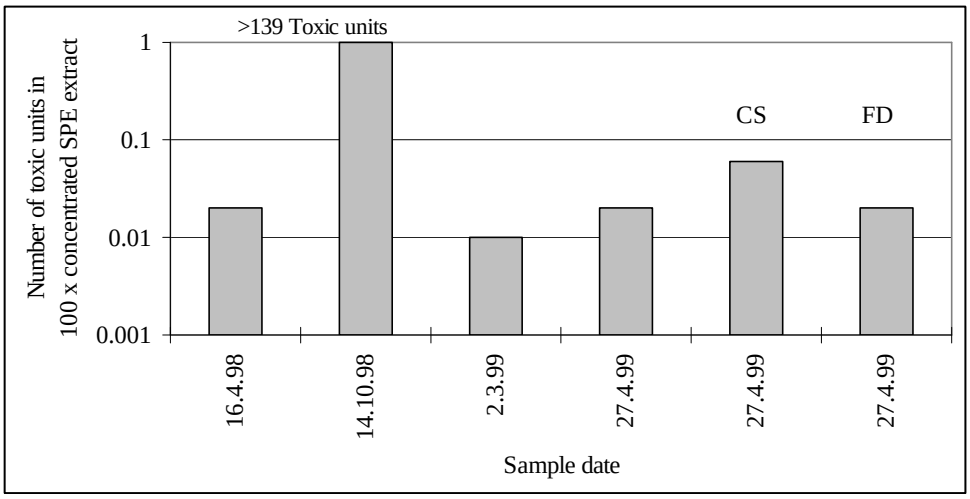

Figure 3.39 Graph showing estimated toxic units in stream, control stream (CS) and field drain (FD) samples collected from site D during storm events. Toxic units are based on the results of toxicity tests of solid-phase extracts using the green alga *Raphidocellis subcapitata.* The blank bars indicate the detection limit for those tests in which no effects were observed, and are derived from the SPE concentration factor.

The presence of one or more toxic units in 100X sample concentrate was measured in SPE samples from Site D in the algal growth bioassay (Figure 3.39). On one occasion, 14.10.98, the sample before concentration produced more than 50% inhibition in algal growth relative to a control. In raw water samples however differences in nutrient content relative to control media may also contribute to reduced growth in the stream sample.

For the *Lemna minor* bioassay of SPE extracts from Site D the results from a number of event samples suggest the presence of approximately 1 µg/L equivalent of photosynthesis inhibiting herbicides (this is illustrated in Figure 3.40). Paraquat is added to all samples, and produces a rise in conductivity. Samples, which also contain photosynthesis inhibiting herbicides, produce a reduction in conductivity as compared to that produced by paraquat alone. The magnitude of the difference between conductivity levels in the paraquat only treatment and those treatments with added SPE extracts, is proportional to the concentration of photosynthesis inhibiting herbicides present. Figure 3.40 shows the results of a *Lemna* bioassay conducted on an SPE extract of a sample collected on the 16.5.97 at Dollymans Farm (site D).

Although conductivity decreases with increased sample concentration factor, only at a concentration factor of 16x and above is the mean conductivity significantly less than that of the paraquat treatment. If it is assumed that the herbicidal activity was due to atrazine alone this would be equivalent to an atrazine concentration of >100µg/l in the 16x SPE sample. This would indicate a raw water concentration of very approximately 6 µg/L of atrazine or other herbicides with a similar mode of action. This compares quite well with the actual concentration of herbicides measured at this time, with diuron at a concentration of 5.7 µg/L likely to have been contributing most of the toxic effect (Table 3.5)

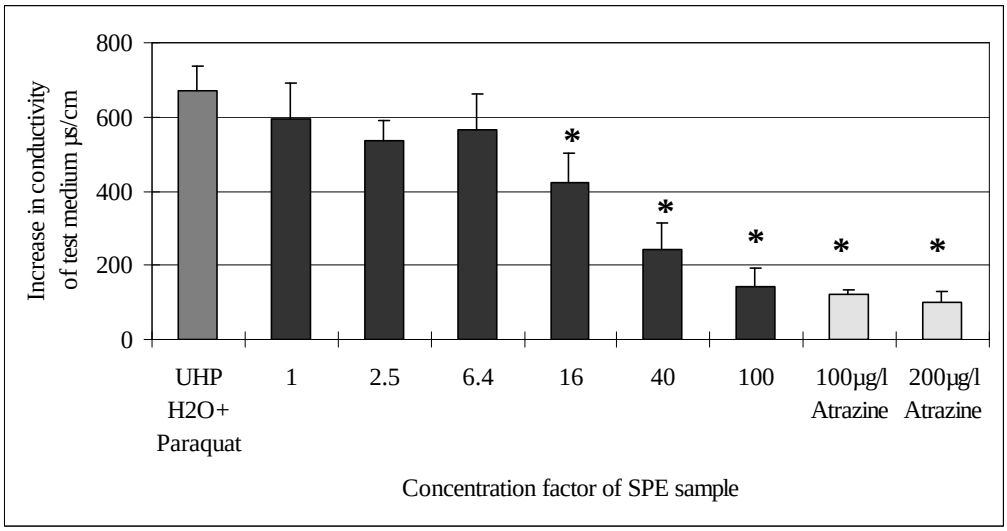

Figure 3.40 Mean (95 percent confidence limits) 48 hour increase in conductivity in *Lemna minor* bioassay of SPE extract of a stream water sample collected during a period of increased stream-flow at site D (16.5.97). The effect of two different concentrations of atrazine on the assay response are also included.

*** these results are significantly different from the paraquat treatment ($p<0.05$)**

.Based on growth inhibition of *Lemna minor* in 7 to 10 day test procedures, the number of units of toxicity for water samples has also been calculated. The results for each site are broadly comparable to those for the green alga *Raphidocellis subcapitata* (Figure 3.26). Samples from May 1997 at Dollymans Farm (site D) contained the highest concentrations of herbicides and hence produced the highest toxic unit values. The highest number of toxic units (based on growth of *Lemna minor*) was measured in an event sample collected on the 10 May 1997. Approximately 97% of the contribution to the toxic unit value of 0.68 was made by the herbicide diuron.

3.4.5 Predicted versus measured toxicity

Figure 3.41. shows the sum of toxic units calculated for the range of compounds measured at site D (Dollymans Farm), based on toxicity studies using *Daphnia magna*. In this case a unit of toxicity is the concentration of one or more compounds required to produce 50% immobilisation of the Cladoceran *Daphnia magna*. The toxicity of each compound to Daphnia was derived from the data presented in Table 3.5. Data in Figure 3.41 indicate that the water samples from site D are of extremely low toxicity to *Daphnia magna*. For most samples the concentration factor required to produce one unit of toxicity falls between 1000 to 100,000. The samples from spring 1997 are of marginally higher toxicity than those taken the following year, falling between concentration factors of 100 and 1000.

The predicted toxicity of the samples at site D was higher for the algal assay (Figure 3.42) than for the *Daphnia magna* bioassay (Figure 3.41). A concentration factor of approximately 100 was required for most samples to produce growth inhibition. Samples collected during spring 1997 however had a relatively higher predicted toxicity since they only require a concentration factor of between 1 and 10 to produce one unit of toxicity.

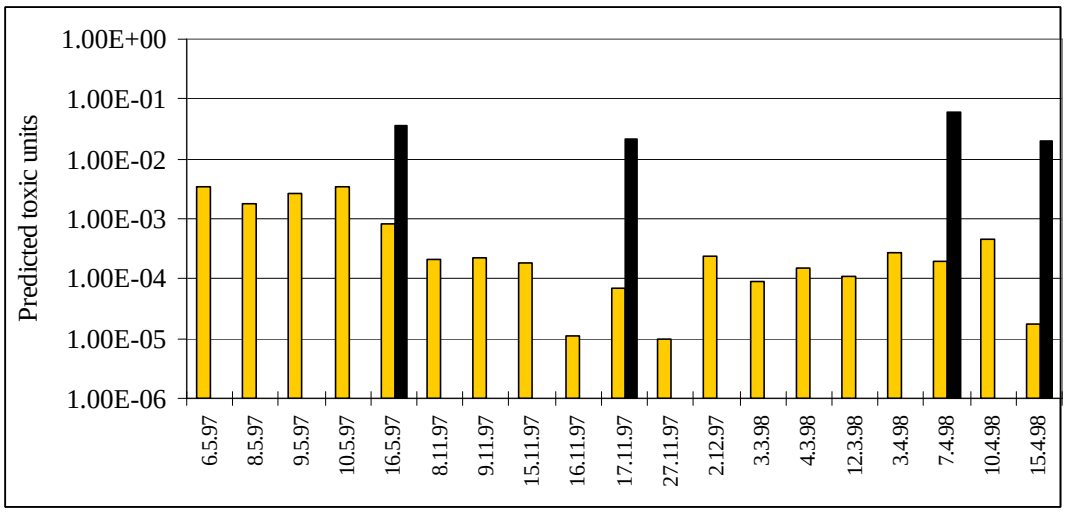

Figure 3.41 Graph showing predicted toxic units(* light bars) for the Cladoceran *Daphnia magna* based on the compounds detected in water samples collected during increased stream-flow, following rainfall at site D. Some measured values are shown (black bars) for comparison.

***To calculate toxic units, the concentration of each compound measured in a water sample is divided by the relevant toxicity figure for *Daphnia magna* from Table 5 and these values are then summed**.

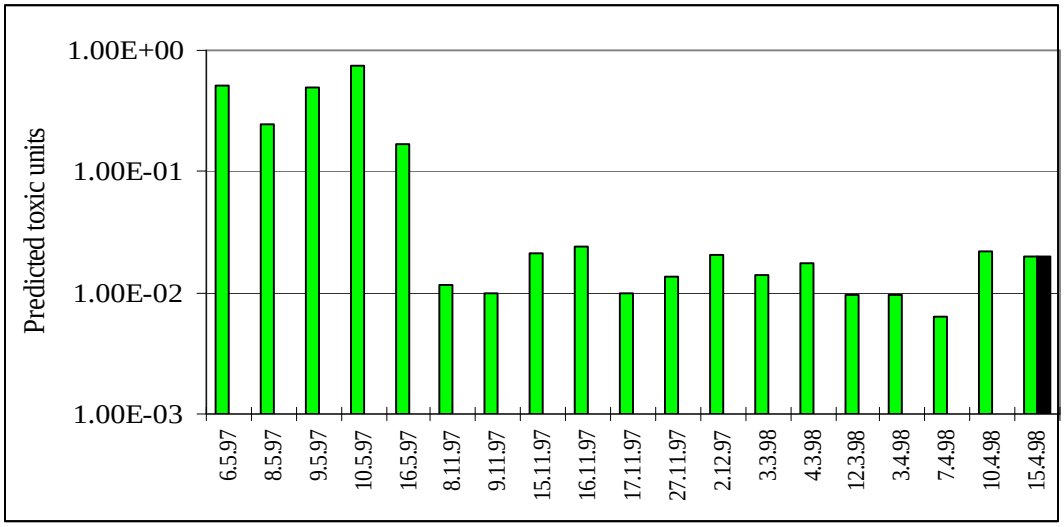

Figure 3.42 Graph showing predicted toxic units(*) for the unicellular green algae Raphidocellis subcapitatum based on the compounds detected in water samples collected during increased stream-flow, following rainfall at site D. The dark bars show the number of toxic units calculated on the basis of the Raphidocellis bioassay of the SPE extracts of stream samples.

***To calculate the toxic units, the concentration of each compound measured in a water sample is divided by the relevant toxicity figure for unicellular algae from Table 5 and these values are then is summed.**

3.4 Indigenous fauna and flora

At Dollymans Farm (Site D) the macroinvertebrate diversity at the main sampling station was very low in both drift and kick samples. Although comparable numbers of organisms were sampled in drift and kick samples at this site, two groups chironomids and oligochaetes alone made up 63 - 99% of the organisms sampled during spring 1997. Between January and early March 1998 Asellus dominated the samples with oligochaetes once again becoming a dominant group as the season progressed.

During February 1998 the numbers of drifting organisms was greatly reduced, this coincided with a period during which high mortalities were also observed in the *Gammarus* bioassay. Spray application of the pyrethroid cypermethrin had occurred at the end of January and may have been linked with these observations.

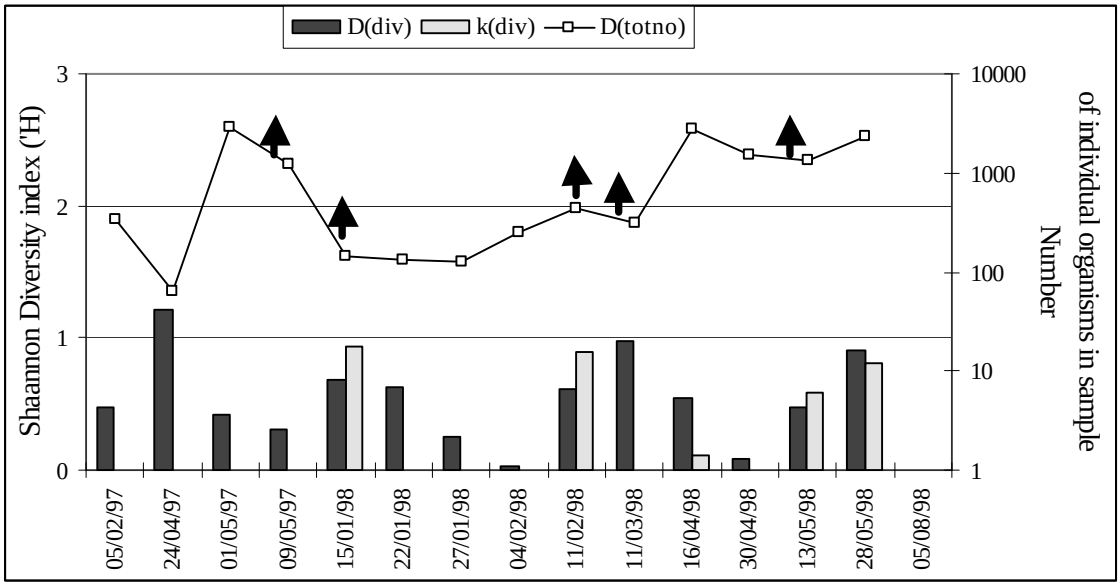

Figure 3.43 Shannon diversity index for macroinvertebrates sampled in drift nets (dark bars) or kick samples (light bars) collected at Dollymans Farm (Site D). The broken line indicates total numbers of organisms in the drift sample. Arrows indicate that a runoff event occurred during the week preceding the date on which a sample was collected.

The highest and lowest Shannon diversity scores for kick samples collected from each of the main sites between 1997 to 1999 are shown in Figure 3.43. Not all of the kick samples were processed, so it is not possible to compare similar sample dates therefore these values can only be considered indicative. Nevertheless the diversity scores for Dollymans Farm (Site D) were much lower than those for the other sites. The frequent observation of toxic effects at site D either related to storm events or associated with pesticide application, strongly suggests that for this site there may be a link between the observed species diversity and the toxic effects of pesticides.

3.5 Summary of results for each of the sites

The number of pesticides applied to site A, C and D during 1997 is shown in Figures 3.44 to 3.49.

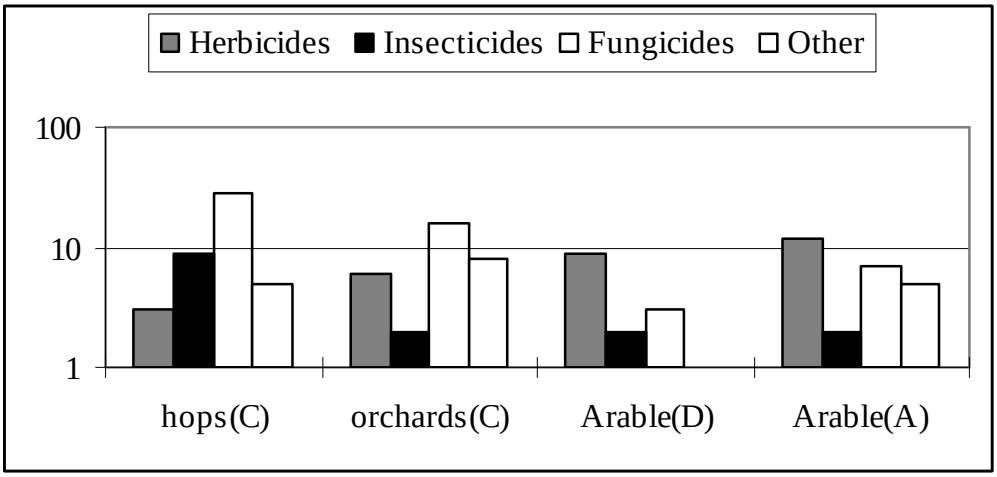

Figure 3.44 Pesticide applications by type for each of the sites in 1997 (values for C refer to a single hopyard or orchard, there are four and three respectively in the catchment that had a similar application record)

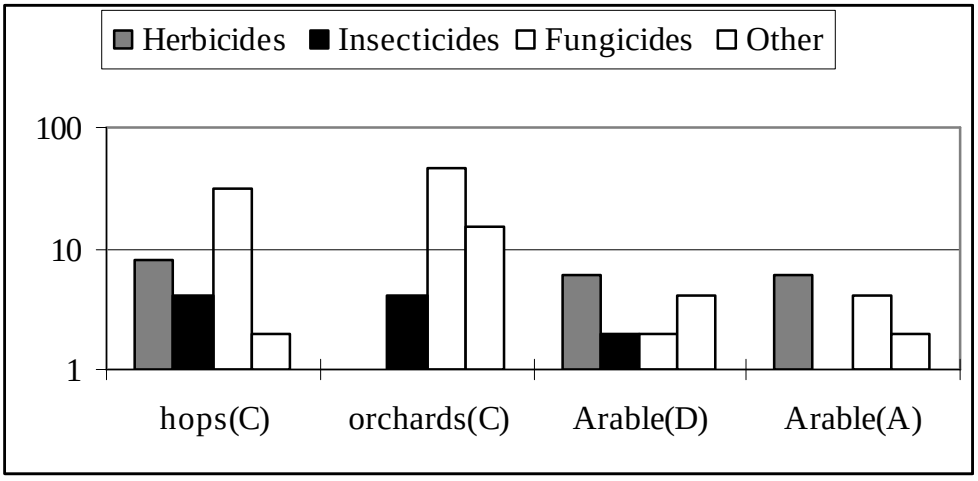

Figure 3.45 Pesticide applications by type for each of the sites in 1998 (values for C refer to a single hopyard or orchard, there are four and three respectively in the catchment that had a similar application record)

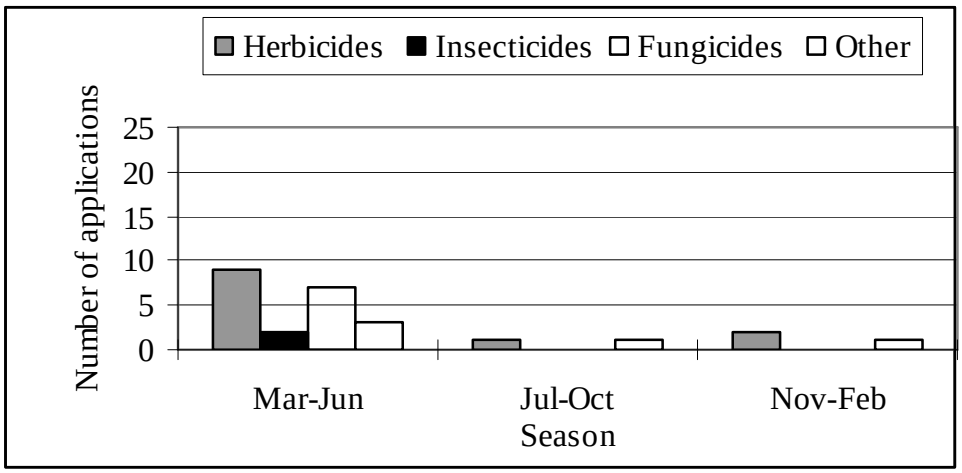

Figure 3.46 Seasonal pesticide applications to the Arable crops on site A during 1997.

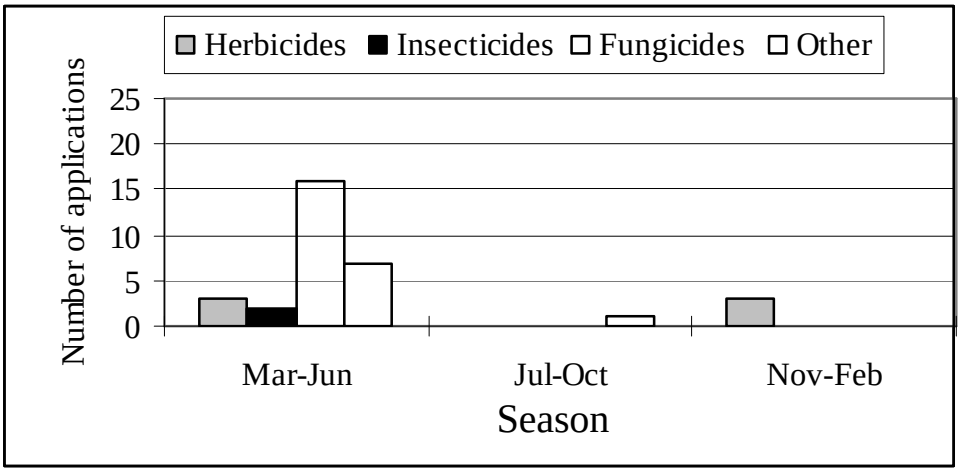

Figure 3.47 Seasonal pesticide applications to the hop gardens on site C during 1997.

The major compounds applied at each of the sites were fungicides and herbicides. The hop gardens and orchards received the largest number of applications over the study period. The pattern and number of applications between 1997 and 1998 for all sites was essentially similar. The main difference was the absence of herbicide applications on the orchards due to the wet autumn weather, which also prevented application of insecticides at Rye Farm (Site A).

Of the 26 compounds analysed for between 1997 to 1999, 12 were detected in water samples collected by the automatic sampler during triggered events. Of these 12, two were fungicides and the remainder herbicides. Selection of compounds for analysis was based on their known application and the likelihood they would leach. Some of the analytical techniques employed were suitable for the detection of a range of related compounds, therefore in some cases pesticides which were not known to be applied were also detected.

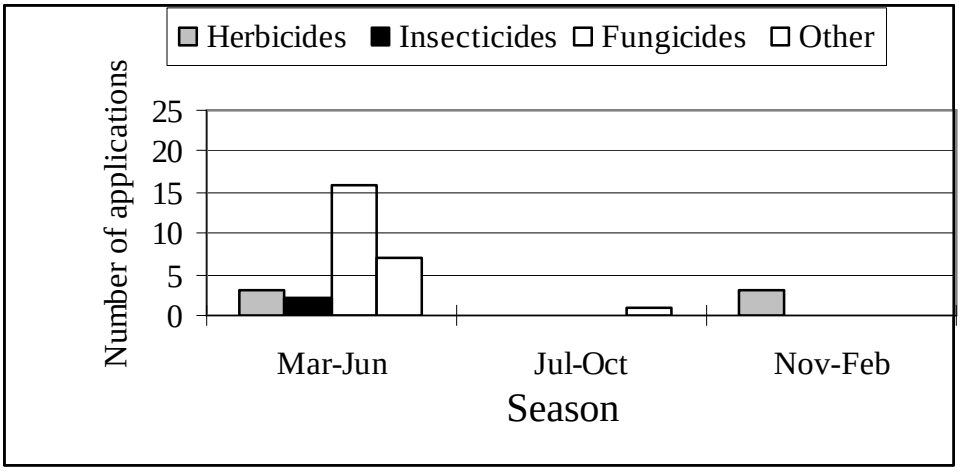

Figure 3.48 Seasonal pesticide applications to the orchards on site C during 1997.

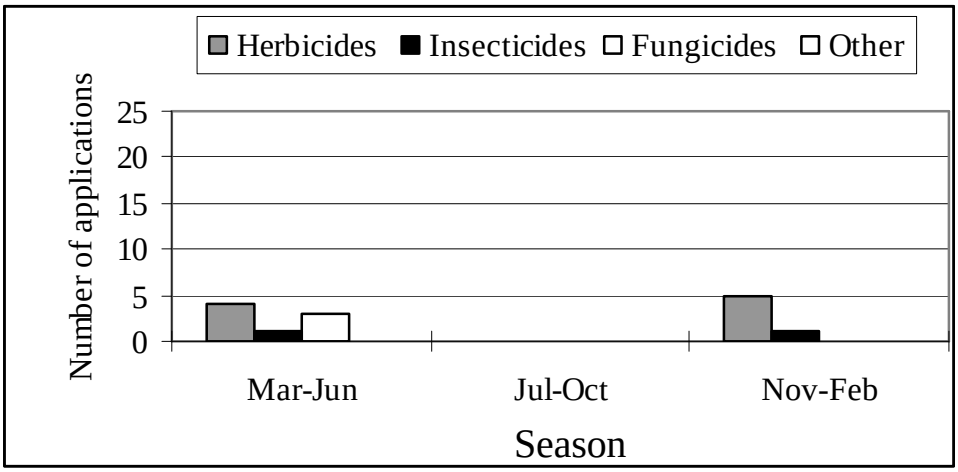

Figure 3.49 Seasonal pesticide application for the arable crops on site D during 1997.

Table 3.10 lists some of the physico-chemical characteristics that influence the occurrence of these compounds in environmental samples. Water solubility, affinity for sorbtion to organic matter (K_{oc}, sorption coefficient, ml of compound per gram of organic carbon) and degradation rate in soil and water are all important factors influencing the probability that a pesticide will be leached from agricultural soil. The groundwater ubiquity score (Gustafson, 1989) uses the soil half life and K_{oc} value in order to calculate a number which indicates the likelihood that a given pesticide will be prone to leach from soil. Since pesticide half-lives for soil and K_{oc} values can be derived from a variety of experimental protocols, predictions of pesticide behaviour must be treated with a degree of caution.

Of the 169 pesticides analysed by the Environment Agency in 1997 isoproturon (17% of records) most frequently exceeded the 0.1 µg/l standard specified in the Drinking Water

Directive for potable waters (EA, 1997). Mecoprop (13%), diuron (12%), MCPA (6%), simazine (5%) and 2,4-D (4%) also frequently exceeded the standard.

Diuron was one of the compounds most commonly detected in this study. Its use was recorded for site C on the hop gardens for general weed control and it is also used in various mixed formulations for control of weeds in orchards. There was no record of diuron application at site D although it was detected on a number of occasions. Although it is used for control of grasses and weeds in cereal crops it is also used for control of weeds in amenity areas and the stream at site D received some road drainage and potentially runoff from hard surfaces in a nearby local town.

Table 3.10. Physical data for each of the pesticides detected in water samples collected from the three sites surveyed during spring and autumn 1997 (all data derived from The Pesticide manual, unless otherwise referenced)

Pesticide	Aqueous solubility (mg/l)	K_{oc} (1) (ml/g)	K_{ow}(2) (log P)	GUS(3)	Overall in soil DT_{50}(4)
Atrazine	33	39-155	2.5	3.27 PL	35-50(6)
Chlorotoluron	74	155(5)	2.5	2.79 TL	30-40
2,4-D	311	60	2.6-2.8	1.88 TL	<7
Diuron	42	400	2.8-2.9	2.99 PL	90-180
Fenpropimorph	4.3	862-4500	2.6	0.99 IL	15-93
Isoproturon	65	130(4)	2.5	2.32 TL	6-28
MCPA	734	20(4)	2.75	2.28 TL	<7
MCPB	44		2.79		
Mecoprop	734	12-25	1.26	2.73 TL	7-13
Propiconazole	100		3.72		40-70
Simazine	6.2	103-377	2.1	3.16 PL	70-110

(1) K_{oc}, , sorption coefficient, ml of compound per gram of organic carbon
(2) Ground water ubiquity score, an index which indicates the likelihood that a pesticide will leach from soil. GUS value = log10 (soil DT_{50}) x (4 - log10 (K_{oc}), PL=leacher, TL=will leach under some conditions, IL= unlikely to leach (Gustafson, 1989).
(3) K_{ow}, is the octanol water partition coefficient
(4) Time taken for 50% degradation of a parent pesticide.
(5) Wauchope *et al*., 1992
(6) Riza, 1996
(7) In instances in which a range is quoted the average value has been used to calculate the GUS.

At site C, diuron was detected in runoff samples from both spring and autumn during 1997 (Figure 3.10), which is similar to the results observed for site D. Of the other pesticides detected in 1997, simazine was present in both spring and autumn samples at sites A and C (Figures 3.3 and 3.10), but was detectable in spring samples only at the Dollymans Farm (site D) (Figure 3.34). Simazine, like diuron, has a relatively long half-life in soil (70-110 days), which will contribute to its persistence in water draining from agricultural catchments. Toxicity data for the compounds detected in samples from each of sites A, D and C during 1997 are reported in Table 3.5

Detectable levels of some of the herbicides (e.g. diuron) were present during low flow conditions at some of the sites. This fact has no doubt contributed to the lower average algal growth seen in some water samples collected between storm events. Figure 3.50 illustrates this point and also indicates that there is large variability between sites in terms of growth of

algae. Samples from site D and its control site produced the lowest average growth overall. These samples also showed the greatest variation in growth between sampling occasions. Mean growth in event samples was not however statistically different to that during base-flow conditions.

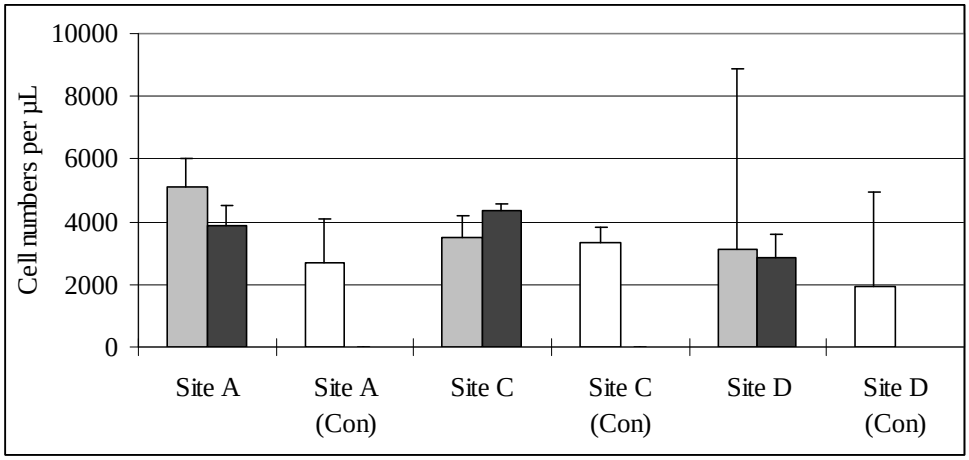

Figure 3.50. Mean (and 95% confidence limit) growth of *Raphidocellis subcapitata* in water samples collected between events (grey bars) event samples (dark bars) and control site samples taken between events (white bars) collected from site A, C and D and associated controls during spring and autumn 1998. All samples had nutrient additions to eliminate this potentially confounding factor.

Algal bioassays conducted on whole water samples present difficulties in interpretation due to the fact that the absence of an essential nutrient or the presence of a toxic compound may both reduce growth. The concentration of toxic compounds in a water sample on a Solid-phase columns offers an advantage in this respect since the concentrated extract that results can be tested in an appropriate standardised medium for the test species.

Figure 3.51 shows the number of toxic units (based on the *Daphnia magna* bioassay) present in water samples from storm events sampled at site C and D. This figure is derived from toxicity tests on the concentrated extract from the solid-phase column. The results indicate that water samples (including field drain samples) showing the highest toxicity still require a concentration factor of >20 to produce one unit of toxicity. At least one third of the samples from site C and D would require a concentration factor greater than 100 to produce one unit of toxicity. The majority of the remaining samples would require a concentration factor between 25 and 50x to produce one unit of toxicity. Factors <1 may nevertheless still imply the potential for sub-lethal effects

Actual toxicity measured in SPE samples from site A (Rye Farm) C (Harpers Farm) and D (Dollymans Farm) was low for *Daphnia* and the green alga *Raphidocellis* (Figures 3.51 and 3.52). The number and magnitude of toxic events was generally comparable for sites C and D. The field drain samples taken at site D were the most toxic sampled, one event on the 7.4.98 produced 6 units of toxicity in a 100x SPE extract (equivalent to 0.06 actual toxic units in the unconcentrated sample).

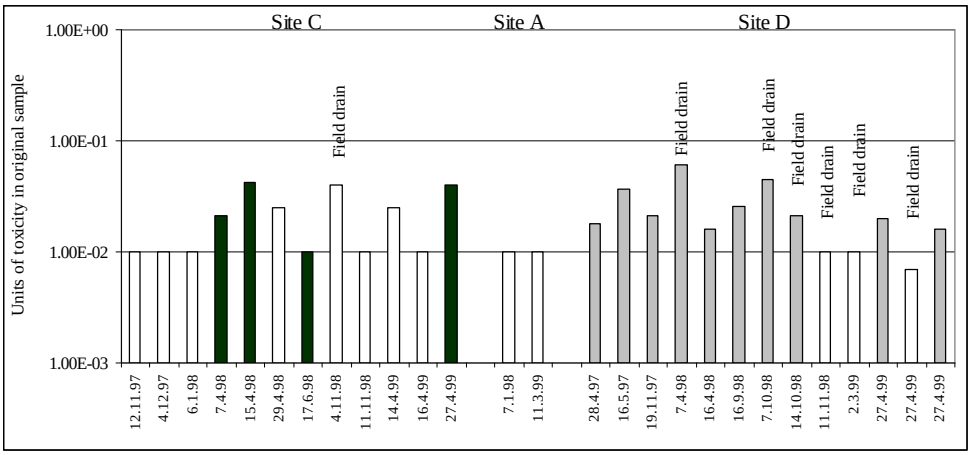

Figure 3.51 Graph showing estimated toxic units in stream or field drain samples collected from site A, C and D during storm events. Toxic units are based on the results of toxicity tests of solid-phase extracts using the Cladoceran *Daphnia magna.* The blank bars indicate the detection limit for those tests in which no effects were observed, and are derived from the SPE concentration factor.

Figure 3.52 shows that toxicity tests of SPEs of samples collected during storm events using the green alga *Raphidocellis subcapitata.*

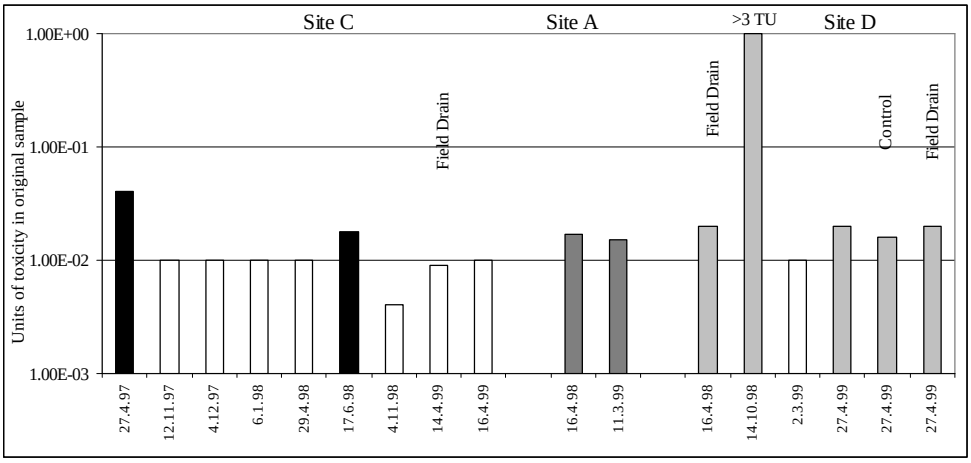

Figure 3.52 Graph showing estimated toxic units in stream or field drain samples collected from site A, C and D during storm events. Toxic units are based on the results of toxicity tests of solid-phase extracts using the green alga *Raphidocellis subcapitata.* The blank bars indicate the detection limit for those tests in which no effects were observed, and are derived from the SPE concentration factor.

The results for the algal assay of SPEs are broadly comparable to those for *Daphnia magna* for the same sites and sample dates. The main exceptions were the sample collected on 14.10.98 from site D in which the sample required dilution rather than concentration in order to estimate 50% growth inhibition. In this particular sample there were the equivalent of three units of toxicity. The A sample collected on 11.3.99 from Site A, and the field drain sample on 27.4.98 from Site D both showed 50% algal growth inhibition at approximately 50x concentration, while the Daphnia bioassay produced no immobilisation at concentration factors up to 100x.

Table 3.11 shows the predicted toxicity of storm events (based on measured pesticide concentrations) during a season at Site C and D. Several factors result in these predictions effectively under-estimating toxicity. It has already been noted that predicted toxicity was based on the pesticides analysed for and detected in water samples that in this study were mainly herbicides. Therefore the predicted toxicity to *Daphnia magna* was generally low. Measured toxicity of SPE samples to *Daphnia magna* was one to two orders of magnitude higher than pedicted therefore the toxicity ranges shown in Table 3.11 can be considered understimates for *Daphnia*. In this study water was sampled over a 24-hour period from the beginning of an event, but most of the pesticides in storm event samples appear in the first few hours therefore the derived concentration factors for an event are over estimates. Values in Table 4.1 for both *Daphnia* and *Raphidocellis* can therefore be considered to be approximately one order of magnitude higher than shown based on the two previous considerations. This would mean that 66% of events sampled at Site C and 74% of the events sampled at Site D during spring 1997 and spring 1998 require a concentration factor of 1 to 10 or less in order to produce 50% inhibition of algal growth. For *Daphnia*, toxicity figures in Table 3.11 can probably be considered to move up by two orders of magnitude to take into account both the differences between predicted and measured toxicity and the fact that concentration factors are over estimates. This means that 38% of events at Site C and 21% at Site D require a concentration factor of 1 to 10 to produce acute toxicity.

Table 3.11 Predicted concentration factor range required in order to produce acutely toxic concentrations of pesticides for either *Daphnia magna* or the green algae *Raphidocellis subcapitata* in sampled storm events. Predicted toxicity values for algae are based on the concentration of individual pesticides measured in a sample.

Site	Species	1 to 10	11 to 100x	101 to 1000x	>1000
C	*Daphnia magna*	none	none	8 events	13 events
D	*Daphnia magna*	none	none	4 events	15 events
C	*Raphidocellis subcapitata*	3 events	11 events	7 events	none
D	*Raphidocellis subcapitata*	5 events	9 events	5 events	none

Multivariate analysis techniques are recommended for the analysis of community data in order to make full use of the information available (the presence or absence of species and their relative abundance) in a sample of stream fauna.

For this study, kick sample and drift data were analysed using nonparametric techniques available in the PRIMER (Plymouth Routines In Multivariate Ecological Research) software package. Species abundance data for discrete samples was compared using an ordination (or mapping) technique, multi-dimensional scaling (MDS). This produces a representation of the community data as a map on which the different samples are represented as single points, the distance between respective points is indicative of the similarity or dis-similarity of samples from different stations, streams or periods of time. Figure 3.52 represents all the kick samples that have been processed for Site C and its control ‘fc’ and Site B and D. Using a procedure for the analysis of the (dis) similarities between sample groups from each site, it was demonstrated that the communities from each were significantly different from each other ($p<0.05$).

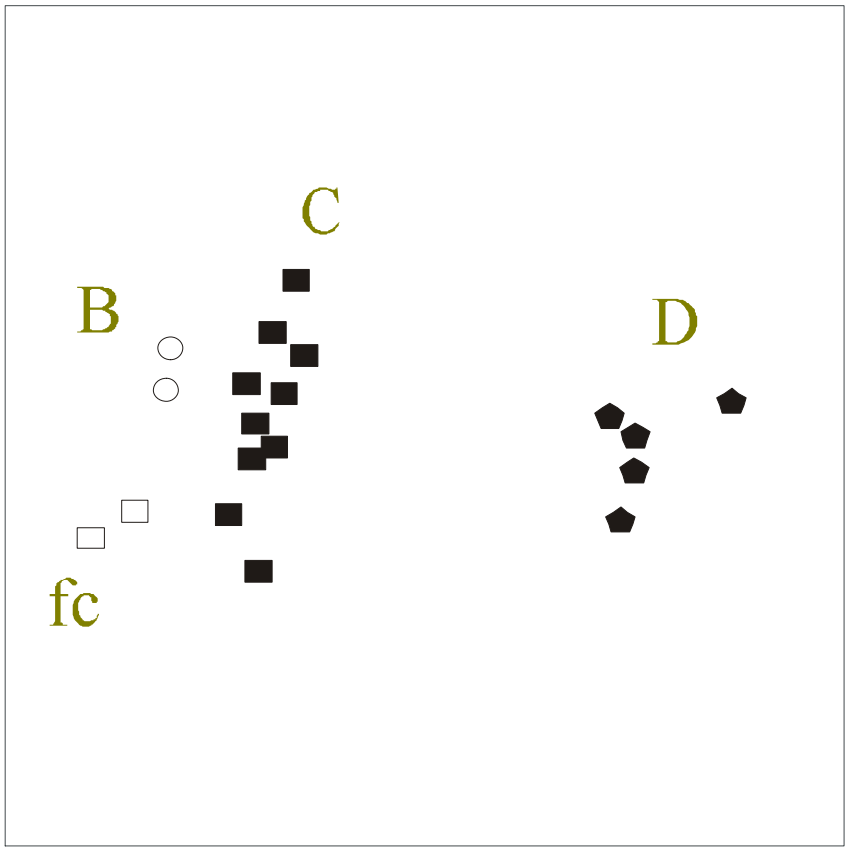

Figure 3.53. Non-metric multi-dimensional scaling ordination of similarity matrix derived from 4th root transformed species abundance data using the Bray-Curtis (dis)similarity index. Samples shown for sites B, C and its control fc and D.

Figure 3.53 shows the results when the same analysis was applied to samples of invertebrates collected in drift samples at Site C between winter 1997 and 1998. In this dataset there is a clear separation of samples by season. This separation was shown to be significant using a one-way analysis by season in the ANOSIM procedure. Those samples that were collected during storm events appear to cluster with other respective samples from around the same sampling period. The one exception is the sample collected on the 4.11.98 which shows a marked separation from the group that it would be expected to be associated with. This drift sample showed the highest species diversity of all the samples represented.

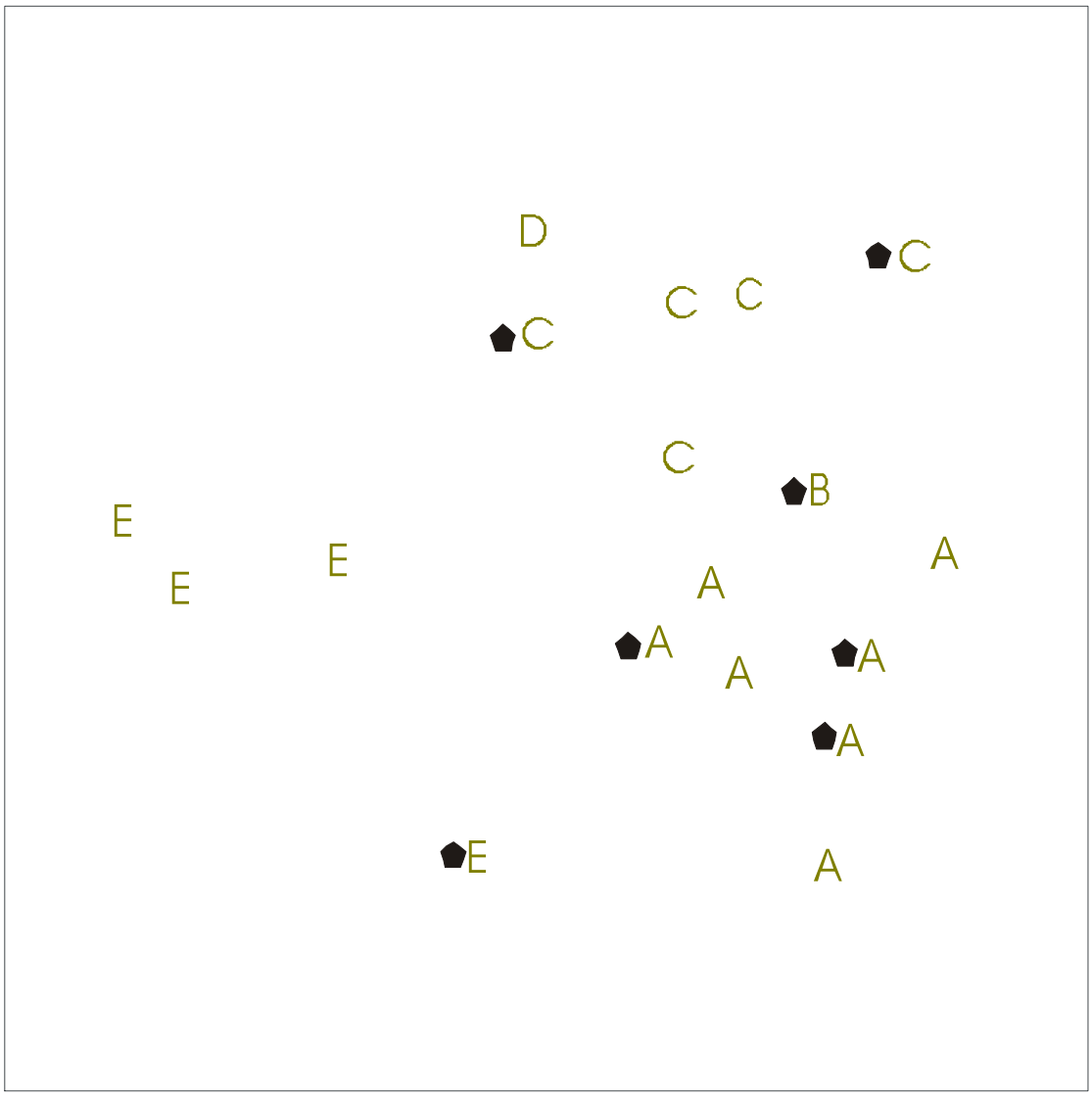

Figure 3.54. Non-metric multi-dimensional scaling ordination of a similarity matrix derived from 4th root transformed species abundance data using the Bray-Curtis (dis)similarity index. Drift samples for Site C: A=October-December 97;B=January-March 98, C=April-June98; D=July-September 98; E=October-December 98. Those samples collected following storm events are indicated by a pentagon.

4. DISCUSSION

4.1 Compounds detected

The 1997 spring/autumn season was characterised by an extended dry period during the spring with a few relatively small rainfall events followed by a summer period in which a series of larger events occurred. The autumn-winter period also contained a number of periods of heavy rainfall that triggered events at each of the sites. This wet period delayed the application of herbicides and aphicides until the spring of 1998. The focus of this study is mainly the spring and autumn events which would normally be expected to produce the highest measurable levels of pesticides (storm runoff) from late autumn and summer applications respectively. In 1997 the higher summer rainfall may have resulted in the reduction of residual soil concentrations of compounds applied during this period. The persistence and mobility of compounds in the soil are likely, however, to have been the most important factors determining the range of pesticides detected.

The largest numbers of pesticides in stream water were measured at the Dollymans Farm (Site D) near Wickford in Essex. These were (as at sites A and C) mainly herbicides, although low levels of the fungicides fenpropimorph and propiconazole were also detected early in the season.

The compound detected at the highest concentration in any of the samples was the herbicide diuron, which was measured at a concentration of 27µg/L at Dollymans Farm during May 1997. Its long half-life in soil (3 to 6 months, Caverley 1983) is probably the main reason for its appearance in water samples at this concentration. This concentration of diuron is close to the concentration (36 µg/L) shown in the laboratory to produce 50% inhibition in the growth of a green algal species (Table 3.5). Exposure periods in most laboratory studies, however, are one to several days and the exposure duration in a storm event in a headwater stream is more typically several hours. Diuron has been shown to be acutely toxic to macroinvertebrates in a concentration range between 0.16 to 0.47 mg/L (EQS), once again following exposure durations of several days. The other herbicides detected at each of the sites were at lower concentrations, the highest levels falling between 0.5 to 5 µg/L. Reference to Tables 3.2 to 3.4 and Table 3.5 suggested that the concentrations of each of the herbicides either singly or acting together (assuming joint additive action) are unlikely to produce acute effects upon the headwater stream flora or fauna. It is however notable at site D (Figure 3.36) that algal growth in raw water samples increased during the spring season in 1998 following a series of storm events. This is most likely related to increased levels of nutrients in the storm waters following applications to various crops. The combination of higher herbicide concentrations together with increased nutrients may well influence the species composition of the attached algal community. If the result of this is to increase the dominance of less palatable species this may have important implications for a number of invertebrate species for which attached algae form a significant percentage of their diet.

Study Site B, the River Tillingham catchment in West Sussex, has intensive sheep rearing on soils expected to leach some pesticides to field drains. Analysis of storm event samples however did not detect the presence of the most common organophosphorus or synthetic pyrethroid active substances used in sheep dip formulations at this site. The macroinvertebrate data also support this finding. Kick samples collected during December

1998 and January 1999 were species rich and included several groups (the crustacean *Gammarus pulex*, stonefly larvae and caddis fly larvae) which would be heavily impacted by the presence of sheep-dip insecticides. It is known that at least some of the farmers in the immediate vicinity of the sampling point on the River Tillingham legally dispose of spent dip to land. The absence of measurable concentrations of sheep dip insecticides in the River Tillingham catchment provides some reassurance that these chemicals are not reaching surface waters as a result of approved management practices. Figure 4.1, which shows the estimated total number of sheep in different parts of the U.K. This Figure demonstrates that despite the fact that relatively smaller numbers of sheep are reared in the South East than other parts of the country, there is nevertheless a high density of animals reared in this region. More specifically 17% of these animals are reared in East Sussex. The total number of animals in the survey area represents 1% of the total for East Sussex.

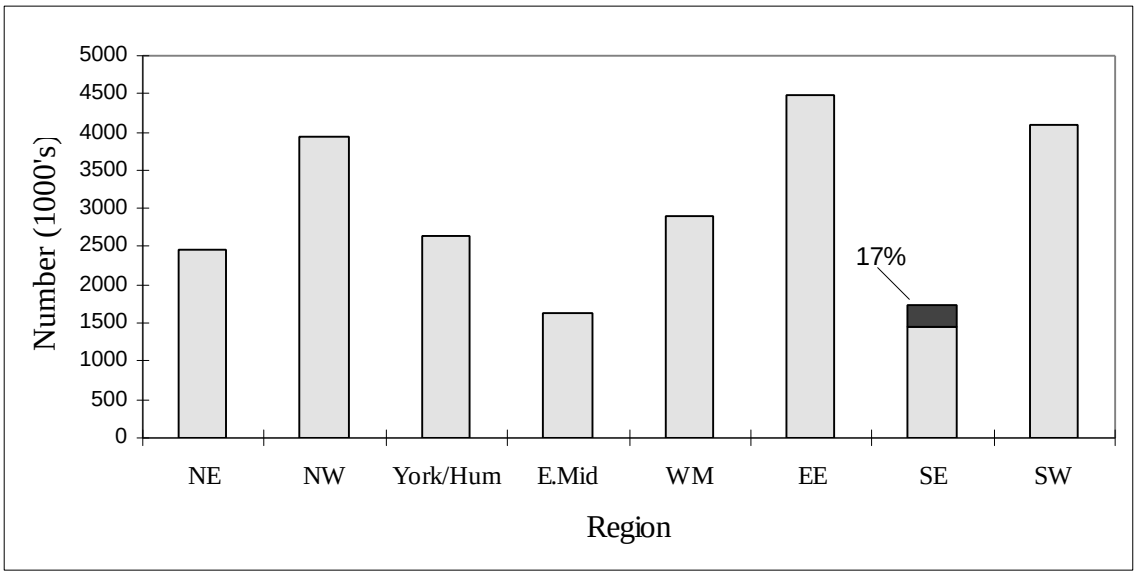

Figure 4.1 Total number of sheep reared in different regions of the U.K. The percentage of the total number reared in the South East (SE) which are reared in East Sussex is indicated.

NE=North East, NW=North West, York/Hum=Yorkshire and Humber, Emid=East Midlands, EE=East of England, SE= South East (includes Kent, East and West Sussex, Oxfordshire and Buckinghamshire), SW= South West

The River Tillingham catchment was chosen for study because of the presence of cracking clay surface geology which is prone to by-pass flow and hence has a greater potential for the translocation of operationally applied (or in this case disposed) pesticide from fields, to nearby streams.

Difficulty in locating land within the catchment on which dip was disposed and in obtaining co-operation with landowners, resulted in an assessment of some of the main sheep-dip pesticides in the River Tillingham itself. The number of sheep grazed in the immediate catchment was high, estimated as 4 to 5 animals per hectare (NRA Project 208, 1994) and therefore the likelihood of spent dip disposal locally was also high. None of the three target pesticides, chlorfenvinphos, diazinon or cypermethrin were detected in stormwater samples taken from the River Tillingham. There are several possible explanations for this result. The

organophosphorus pesticides are relatively rapidly degraded, the DT_{50} for diazinon in laboratory studies was 11-21 days, in soil (The Pesticide Manual, 1994). Other studies have reported a DT_{50} of 79 hours in natural water (Ferrando *et al*., 1992). Cypermethrin is relatively more persistent in soil, complete hydrolysis reported within 16 weeks (The Pesticide Manual, 1994). cypermethrin is also strongly adsorbed to particulates and its half life in the water is reported in a number of studies as appoximately 12 hours (Hill *et al*., 1994). Because of the affinity of cypermethrin for adsorbtion to particulates, the hydrosoil in aquatic systems is the major sink (Heimbach *et al*., 1992) Therefore one explanation for the absence of detectable levels of the major sheep-dip pesticides may be the fact that these compounds are strongly bound in the soil at the site of disposal and that significant degradation took place before sufficient rainfall occurred to flush them into streams draining the catchment.

A number of studies have however reported considerable movement of pesticides with high potential for adsorption to soil particles, in drainage water in agricultural catchments. This enhanced mobility occurs due to the fact that many non polar organic contaminants also bind to colloidal material in soil (e.g. humic substances and microorganisms) and these colloids may become mobilised in groundwater as a result of geochemical and biologic processes (McCarthy and Zachara, 1989, Worrall *et al*., 1994). Studies have demonstrated concentrations of up to 0.6µg/l of diazinon and 1.6µg/l of propetamphos in a small stream 400m from an underground soakaway 2-3h after disposal has occurred (Littlejohn and Melvin, 1991). In the present study once dilution had taken place in the smaller streams feeding into the River Tillingham comparable levels of diazinon to those reported by Littlejohn and Melvin, would be likely to fall below the level of detection (<0.11 µg/l). The biological diversity data for the station sampled on the River Tillingham was however relatively high. This suggests that biologically significant levels of contamination with sheep-dip pesticides did not occur at the sampling station in the River Tillingham during the study period.

Many of the more serious pollution incidents with spent sheep-dip resulted from poorly managed dip baths immediately adjacent to streams. The switch to pyrethroid insecticides prompted by concerns about the health hazards of organophosphate-based products has also contributed to the increase in pollution incidents involving spent dip. The fact that pyrethroids are less toxic to mammals (acute oral LD_{50} 100->4000mg/kg^{-1}) led to the misconception that they were also less hazardous to the environment. The acute toxicity of cypermethrin to invertebrates ranges between 0.01->2000µg/l (Hill, 1989) for diazinon this range is 0.03->10000µg/l (Giddings *et al*., 1996). Pollution incidents involving spent sheep-dip resulted in the loss of many invertebrate taxa from stretches of 10's of kilometres of streams and rivers, generally in upland regions in the North of England and Scotland (Ends, 1996). The current situation has changed in a number of respects: The EC directive on groundwater protection has required disposal of dip to become subject to the waste management licensing regime. The regulations will oblige farmers to apply for authorisations to discharge and will outlaw disposal by soakaway. There will also be a requirement for farmers and/or mobile dip operators to include information relating to the soil type, geology and hydrogeology of a proposed disposal site (ENDS, 1997, EA technical report P237, 1999). These measures together with the raising of awareness of the pollution potential of dip-chemicals should result in a reduction in the number of incidents. With the requirement for notification of dip-disposal more monitoring can be targeted on specific sites (which was not possible in this study) and this is recommended, particularly in catchments in which the surface geology is subject to by-pass flow.

4.2 Non storm event related toxicity

There were at least two instances of toxicity at Dollymans Farm (Site D) which could not be directly associated with storm events. During April 1997 the feeding rate of *Gammarus pulex* deployed in the stream at Dollymans Farm (Site D) was reduced and subsequent mortalities occurred (Figure 3.13). The organochlorine insecticide lindane had been sprayed in the adjacent field immediately prior to these observations. Lindane was not detected in storm event samples six days after Lindane application (6.5.97), however a storm event sample taken on the 26.4.97 which was not analysed for pesticides produced the equivalent of 0.02 units of toxicity in a *Daphnia magna* bioassay (Figure 3.25). The 48 hour EC_{50} for lindane to *Daphnia* is 485 µg/L (Macek et. al.,1976) therefore if all the measured toxicity was due to lindane this would be equivalent to 19.4 µg/L lindane in the original sample. A number of studies have measured lindane concentrations in stormwater runoff, the reported maximum values ranging between 0.07 to 0.27µg/L (Rosemaund 1987, Liess et. al., 1999). It appears unlikely that lindane was present in the original sample of stormwater at a concentration as high as 19.4 µg/L and was therefore not responsible for the majority of toxicity observed in the *Daphnia* bioassay. The relatively low toxicity of the storm event samples also suggests that other factors largely contributed to the observed *Gammarus* mortality.

The 48 hour LC_{50} value for lindane to *Gammarus fasciatus* is reported as 39 µg/L (Macek et. al.,1976). The application rate for the formulation Gammacol, from which the lindane is derived was 0.5 kg of lindane/Ha. The European drift model suggests that for low crops spray-drift at 5 metres will be approximately 0.6% of the application rate. Recent studies have shown, however, that dependent upon wind speed and the nozzle type of the sprayer, spray-drift at a rate of as much as 7.2% can occur (De Snoo and De Wit, 1998). If this worst-case rate of drift occurred then a toxic concentration of 36 µg/L of lindane would have resulted in the stream. The exposure period would however not be more than a few hours at most, since dilution would occur rapidly. This suggests that lindane spray-drift contamination could have occurred, or that coincident factors were responsible for the mortality observed at this site during this period.

On a second occasion at Dollymans Farm (Site D), during January 1998, high mortalities of caged *Gammarus* (70%) were recorded. This increased mortality occurred in the week following the application of the pyrethroid insecticide cypermethrin. No storm events were sampled during this period. Because no event samples were collected, cypermethrin was not analysed for in stream water, also little transport of bioavailable material would have occurred via the field-drain . The extremely high toxicity of this compound (96 h LC_{50} 0.1 µg/l, for *Gammarus pulex*, Hill, 1989) makes it likely that spray-drift may have been responsible for the observed mortality in the deployed *Gammarus*. In the absence of supporting analytical data, however, this mortality can only be considered co-incidental to cypermethrin application.

A number of other rainfall events were recorded at Dollymans Farm which did not produce associated mortality - although on several occasions the stormwater was discoloured immediately after the application of slurry to the adjacent land. There was also a problem with leakage from a slurry lagoon during 1999, this incident is currently being dealt with by the local Environment Agency. Figure 4.2 shows rapidly decreasing oxygen saturation in the stream presumably over the period in which the leakage occurred. Macroinvertebrate samples

were collected during this period but have not been processed. On one other occasion the water was coloured blue by unknown substances.

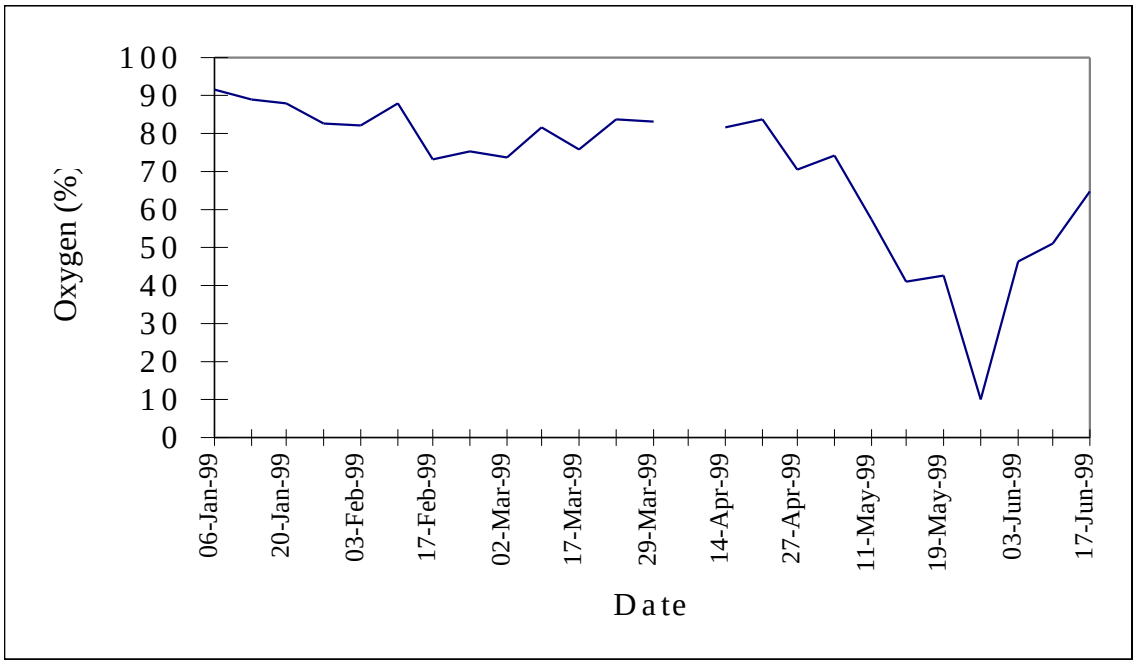

Figure 4.2 Weekly readings (taken between 1200-1400) of percentage saturation dissolved oxygen at Dollymans Farm (Site D) during 1999.

The proximity of this site to a light industrial area and housing was clearly responsible for some of the observations made during the study period. Many farms in the South of England, however, are in close proximity to residential and light industrial areas and therefore Site D is not atypical.

Over the three-year period of the study some channel clearance also took place at Dollymans Farm. This practice is intended to prevent flooding but can be extremely disruptive for the establishment of a diverse macro-invertebrate community. This is an important point to note since there may be many factors in addition to pesticide application, which can have disruptive effects upon headwater stream communities. This latter point was noted in the Environment Agency study of impacts upon headwater streams (Furse *et al*., 1995a).

4.3 Predicted versus measured toxicity

In order to identify potential sources of impact upon headwater communities that are specifically associated with pesticides, initial predictions of toxic effects were made based on those compounds detected during storm events. The actual toxicity of compounds was also measured using various bioassay techniques applied to concentrated extracts of water samples from storm events. Figure 3.38 and 3.39 show the predicted toxicity of the compounds measured at Site D to the green alga *Raphidocellis subcapitata* and the crustacean *Daphnia magna*. These data indicate that predicted toxicity is higher for algae than for *Daphnia magna*. This is not unexpected since the majority of compounds detected were herbicides that are specifically developed for their phytotoxicity. The bioassay of the water samples collected following storm events provides a means of comparing the predicted toxicity to that measured

in a given bioassay procedure. If the same species is used for both the toxicity prediction and the bioassay then the two toxicity values should be very similar. Lower bioassay toxicity than predicted may indicate that there are antagonistic effects between the compounds known to be present in a sample. Higher toxicity than predicted may indicate that some of the compounds present act synergistically with others to enhance the overall toxicity of a sample, or that undetected toxic compounds are also present. Synergism is known to be a relatively uncommon phenomenon, and the presence of other unmeasured compounds has a high probability in samples collected from streams draining agricultural catchments. In the present study only a small percentage of the actual measured toxicity to *Daphnia magna* was predicted for SPE samples on the basis of compounds known to be present. More detailed chemical analysis of one of these samples identified nonylphenol and the insecticide endosulfan sulphate as contributing to the observed toxicity.

The two main bioassay procedures used to identify toxicity in event samples both measure acute toxicity and therefore do not evaluate the potential for longer-term population effects. Although summed toxic units were close to a value of one during certain events (Table 3.11), these values were derived on the basis of 24 to 72 hour exposures. Exposure durations in headwaters are often extremely short (e.g. several hours). Therefore the potential impact upon headwater communities of these transient peaks of pesticides which occur in storm events needs to be considered both in terms of the infrequency of occurrence and the likelihood of sub-lethal effects.

In catchments for which the surface geology is predominantly cracking-clay the results of this study indicate that headwater stream fauna may be exposed to an average of six storm events in a season which would require a concentration factor of 1 to 10 to produce acute toxic effects in exposure periods of 24 hours or more (Table 3.11). For the algae an average of fourteen events in a season would require a concentration factor of 1 to 10 or less to produce acute toxic effects in exposure periods of 24 hours or more. A number of other studies for the U.K. have reported the occurrence of up to five rainfall events (generally of 10 mm/day) which produce measurable inputs of insecticides in headwater streams during a year (Williams *et al*., 1995).

It has already been noted that exposure in headwater storm events is generally in the order of five or six hours. This makes extrapolation of toxicity test results from studies with typical exposure periods of between 24 to 96 hours difficult. Based on the bioassay period used for *Daphnia* and *Raphidocellis* (48 and 72 hours) the exposure period in the headwater stream is equivalent to between 12.5 and 8% of the respective EC50s for each species. The standard approach to measurement of toxicity in the laboratory involves the measurement of mortality or other endpoint for organisms which are continuously exposed to a toxicant. A number of studies have demonstrated that similar levels of mortality may result after shorter exposure periods than those traditionally adopted in continuous exposure designs (Pascoe and Shazili, 1986). In this case irreversible toxic effects may have taken place within the initial few hours of the exposure period. It is also important to note that in standard test procedures mortality, which occurs following the standard exposure period is not, recorded. Post exposure mortality clearly may be of great significance and some authors have suggested that post exposure periods be incorporated into toxicity test designs (Abel, 1980 and Pascoe and Shazili, 1986).

One further point requiring comment in the present study is the presence of nonylphenol in a sample extract from Site C. The HPLC fraction of the SPE extract in which the nonylphenol was identified contributed the most to the measured toxicity. Based on the concentration factor of the SPE extract the approximate concentration of nonylphenol in the original sample was estimated as 8 µg/l. This concentration exceeds the provisional U.K. Environmental Quality Standard for this compound for which the annual average figure is set as 1µg/l and the maximum allowable concentration as 10 µg/L (Wilkinson et al., 1997). This compound is not present in household detergents and so is unlikely to have been derived from this source. Many adjuvants used to facilitate the dispersion of pesticide formulation and increase foliar penetration of herbicides do however contain a range of surfactants some of
these are alkylphenol ethoxylates of which nonylphenol is a breakdown product. Nonylphenol is relatively more toxic than the longer chain ethoxylated parent compounds and also has oestrogenic properties. This compound has also been reported as a present at biologically significant concentrations in other studies focussing on agricultural drainage water (Johnson *et al*., 1998). The ability of surfactants to increase membrane permeability and also solubility of a range of other compounds also suggests that the frequency of occurrence of this compound in agricultural drain water in the U.K. should be given further consideration.

4.4 Field drain toxicity

Toxicity was demonstrated to *Daphnia magna* in two of the five field-drain samples collected from Dollymans Farm (Site D) (Figure 3.25). On the 16.9.98 stream water sampled from Dollymans farm produced toxicity at a 50 times concentration, at this time flow was not sufficient to trigger the autosampler at the site. In the following week there was moderate flow from the field drain but stream flow was again not sufficient to trigger the autosampler. The sample from the field drain at this time was acutely toxic to *Daphnia magna* in a 48 hour test period. The toxicity of this drain sample was estimated as equivalent to three units of toxicity. It seems likely that moderate rainfall during this period produced some flow from the field drains and that the toxicity observed in the stream observed earlier probably originated from the field-drains at this time. In the following week after the field drain sample was taken manually a drain sample was automatically taken during a triggered event in the stream (14.10.98). The toxicity of the drain was considerably lower as a result of the increased dilution occurring following heavy rainfall. The toxicity observed in each of these examples could not be directly linked to the presence of pesticides. To our knowledge no recent applications had taken place in the catchment, the most recent applications being over two months earlier. Some slurry spreading had occurred around the period in which toxicity was observed and there appeared to be a greater probability therefore, that this was responsible for the observed toxicity. The presence of toxicity in the stream at Dollymans Farm was also measured on one occasion when the field-drain was not shown to be producing toxicity. Insufficient samples were collected from field-drains to enable a comprehensive comparison between toxicity of drain-flow and in stream toxicity however a number of instances of toxicity for Site D were not pesticide related and those that potentially were, are likely to have resulted from spray-drift contamination.

4.5 Potential for community level effects

The toxicity of storm events in this study was estimated using standard laboratory test procedures.

Table 4.2 Factors modifying the response of organisms to toxic compounds in laboratory studies and in natural communities.

Modifying factor	Influence in laboratory study	Influence in natural community
Age	Usually standardised (all same age)	All age groups represented some wide range of susceptibility
Nutritional status	Optimal culture conditions may or may not be fed during test period	Variable, in times of seasonal limitation may be more susceptible feeding may enhance uptake
Reproductive status	Mainly non-reproductive unless chronic test procedure	All stages of reproductive status dependent upon season
Health status	Disease free	Disease and parasitism, variable.
Water quality	Standard medium	Variable hardness, pH and other potentially modifying factors
Exposure	Single exposure for standard time period	Often multiple and mostly brief
Species interaction	Often a single species study	Many species, different sensitivities, different trophic level interactions.

A wide range of factors that do not operate in standard laboratory studies, however, may modify the response of a community of organisms to such events. Some of the factors modifying the response of organisms to toxic compounds are outlined in Table 4.2.

In an attempt to evaluate the significance of the exposures that occur in headwater streams the factors outlined in Table 4.2 must be considered. The most easily measured factor in laboratory studies is mortality. In a field situation it may be difficult to measure mortality of organisms in a community but indirect measures such as increased tendency to drift or of mortality of caged organisms can be of value. In the present study high mortality of caged *Gammarus* was recorded at Dollymans Farm on several occasions. On many occasions *Gammarus* were not sampled at this site in kick samples but at other times were moderately abundant. Species diversity was low although groups such as chironomids and oligochaetes as well as Asellus and Sphaerium were relatively abundant. There was sufficient evidence at this site to suggest that there were several episodes of toxicity each season in the stream which were not directly related to field drainage but which produced significant mortality of particular macroinvertebrate groups.

Use of multivariate techniques discriminated significant differences between the invertebrate community present at site C and its control and between sites B and D also. In general the community at D was the least diverse although sites C and D were generally comparable in most other respects.

In the Curtisden Green stream, species diversity was highest in the upper catchment during the early part of 1998 but was reduced following a period in June in which there were two events of increased stream flow and measured toxicity in the *Daphnia* bioassay. The main groups absent from the June samples were the nemouridae, leuctridae, polycentropidae and helodidae.

Similar results have been demonstrated by Schulz (1999) following surface runoff of parathion and fenvalerate in association with sediments. Even though pesticide concentrations in storm events in headwater streams may reach acutely toxic levels on occasions the duration of exposure is typically short. The drift response in many invertebrate species, however, has been demonstrated to occur at highly elevated levels (increased by a factor of 2,400 in one example, Sibley *et al*., 1991) following brief exposure to contaminants in storm water. Both insecticides and herbicides have been demonstrated to produce increased macroinvertebrate drift (Dodsall and Lehmkuhl, 1989 and Thompson *et al*., 1995) although the relative toxicity of pesticides is often far higher.

Loss of species from stations near the top of the headwater stream is more critical since there is no potential for recolonisation by downstream drift. Although *Gammarus*, stonefly mayfly and caddisfly larvae, may be of similar susceptibility to a range of insecticides in storm runoff, the inability of individual species to rapidly re-colonise a stretch of headwater stream will ultimately determine the severity of the impact of pesticide contaminated storm water upon a population. Field experiments have demonstrated that experimentally induced low oxygen concentrations in a headwater stream reduced the number of macroinvertebrate taxa from 30 to 14 (Edwards *et al*., 1991). Recovery in the number of taxa to pre-exposure levels did occur within 6 to 12 weeks, however this period of disruption may be critical in the life-cycle of a number of species.

In the present study although there were often increased numbers of organisms present in the drift-net samples following a storm event there was no evidence to suggest that elevated numbers of particular species occurred at this time relative to periods between events. The MDS plot for the drift samples from site C did however show evidence of a seasonal pattern. Because the drift net in the Curtisden stream at Harpers Farm, was located at the bottom of the catchment, organisms drifting in response to localised high concentrations of pesticides in storm events may become re-attached to the substrate (or caught up in vegetation) just downstream of where they were dislodged. In addition a number of organisms have diurnal patterns of natural drifting, therefore because drift net samples could only be retrieved on a weekly basis the resolution of event related drift may have been reduced by natural drift. Several species were present in higher numbers at different stations in the Curtisden Green stream in situations in which the habitat was essentially similar. *Gammarus* was present in very low numbers at stations 1, 2 and 4, coincidentally these stations are adjacent to a hop garden in the case of 1 and 4 and an orchard in the case of 2. The other stations are either at intermediate positions between hop yards or orchards or above the influence of runoff from these crop types. A previous study during 1992 to 1993 also looked at similar sampling stations on Curtisden stream, and recorded total mortality of *Gammarus pulex* held in cages at stations 1 to 4 during August 1992 and station 2 (which is just downstream of one of the orchards) during March 1993 (Crane *et al*., 1995). Both periods are coincident with the main application period for insecticides in orchards. None of the previous examples however, can be directly linked to pesticide contamination of the stream because storm events were not monitored.

It is apparent from this study and a considerable number of previous studies that the major difficulty faced in field monitoring programmes is the discrimination of low-level effects of contaminants on ecosystems from natural variation. In studies of headwater streams mapping the number of potential inputs which may influence biological quality is also a problem.

Work is however progressing on the development of assessment techniques for headwater streams which are similar to those used for national River Quality Surveys (Furse *et al*.,1995). Furthermore the development of the a predictive model for quality assessment of headwaters as a module of the current RIVPACS system will allow expected and actual biological quality of a headwater to be compared. This in turn will allow impacted sites to be identified.

Although the use of the Biological Monitoring Working Party score for macroinvertebrates, which is widely applied to lowland rivers has much to recommend it, there is nevertheless a loss of important information due to the reduction of the data to a single score.

Sites that exhibit poor biological quality in seasonal surveys should be examined in more detail. In addition information from impacted sites for which the source of impact is known (e.g. a pesticide spill or over-spray) should be used to provide baseline data sets against which to compare more subtle changes at other sites.

Only the stream fauna has been considered in the preceding discussion however it is only one part of the stream community. It is noted in this and many similar studies that the herbicides predominate in stormwater samples. The aquatic flora consists of the larger macrophytes, which are most commonly rooted, in the sediments, and the algae which may be attached to surfaces or suspended in the water column. These general growth patterns may have a significant influence upon the route of exposure to toxic contaminants and as well as the degree of exposure. Species sensitivity may vary greatly even amongst the algae. The sensitivity of 56 strains of algae, representing seven taxonomic groups, exhibited a wide range of sensitivity to the triazine herbicide simetryne, with values of 6.5 to 1500 µg/l (Kasai et al., 1993). Similarly large differences in sensitivity to herbicides are shown between the algae and higher plants. In addition rooted macrophytes may take up contaminants from the sediments.

In the current study the results indicate that plant communities in headwater streams may be exposed to approximately 12% of the toxic dose required to inhibit growth by 50% over an exposure period of three days. Pulsed exposures of atrazine in mesocosm studies produced reductions in biomass (up to 67%) due to the loss of some species of chlorophyte (Herman *et al*., 1986) therefore it is not unlikely that some negative effects may result in headwater streams exposed to pulses of herbicides. Some negative effects are not necessarily obvious. For example, doubling of chlorophyll content was produced in a mixed microbial community (including algae,bacteria and protozoa) exposed to 3.2 to 10 µg/l atrazine, but this increase was not matched by a greater rate of primary production (Pratt et al., 1988).

Indirect effects of pesticides may also be significant. Application of the organophosphorus insecticide chlorpyriphos to mesocosms at concentrations of five and 35µg/l reduced invertebrate numbers, which in turn resulted in an increase in periphytic algae attached to rooted macrophytes leading to an ultimate reduction in the macrophyte biomass (Brock *et al*., 1993). Uptake and bioconcentration of contaminants is also important since this may result in biomagnification of compounds in the stream food-chain. Atrazine uptake has been demonstrated to be very rapid in a range of freshwater algae and diatoms, 90% of the total uptake occurring within the first hour of exposure (Tang *et al*., 1998). This is significant for pulse exposures that are typically short, and indicates that diatoms and other algae, which are important food items for a range of grazing invertebrates, may play a part in the transfer of contaminants through the food-chain.

Diatom assemblages have often been used in water quality studies, most commonly as indicators of trophic status or organic pollution (Fritz et al. 1993, Christie and Smol 1993, Lange-Bertalot 1979). These studies concentrated initially on lentic systems, where the accumulation of diatom valves on the surface of Lake Sediments leads to a characteristic species composition over different time periods. However in recent years studies have explored the relationship between habitat, and other environmental variables, and diatom distribution (Pan et al. 1996, Kutka and Richards 1996). This allows data from the less temporally stable stream systems to be used with greater understanding. River water-monitoring studies have concentrated on trophic status (Round 1991); biological indices being developed with organic pollution in mind (Kelly et al. 1995).

Diatoms are studied because they are pollution sensitive (Pan et al. 1996) and because the epilithic and epiphytic forms (attached to stones and plants, respectively), attach securely to artificial substrates, such as glass slides or unglazed tiles, allowing control of colonisation period, habitat type and sampling.

Insufficient data was collected in this study to enable an evaluation of the effects of storm events upon attached communities of algae. Attached communities of algae however represent an important resource in headwater streams (Mayer and Likens, 1987), therefore they should be included in monitoring programmes. They are ideal for monitoring because they are attached and have no means of immediate migration and therefore cannot avoid pollution events. They also have relatively short life-cycles therefore rapid responses to the effects of contaminants are possible. Samples are also relatively easy to handle and take up little storage space. As a group attached algae have been used effectively to monitor the effects of a wide range of point-source inputs (Morgan, 1987; Biggs, 1989). The incorportaion of this group in future monitoring programmes is therefore recommended. In addition a small number of laboratory studies to evaluate species sensitivities to the dominant pesticide groups detected in surface waters will enable the prediction of likely shifts in species dominance which will occur as a result of exposure to pesticides in storm events.

Accounting for all the factors that may contribute to the response of aquatic communities following exposure to pesticides in stormwater is a major challenge. There are however extensive bodies of ecological data on a wide range of species which should enable the species most at risk from exposure to pesticides due to their life-history and ecological niche to be identified. The development of predictive models needs to focus on three main areas, chemical fate, exposure and toxicity. The fate of a wide range of pesticides in the environment has now been well described and a variety of models exist to predict fate in soil, groundwater and surface water (Capri and Trevisan, 1998).

Exposure in headwaters is likely to be transient therefore extrapolation from the effects observed in standard continuous exposure tests in the laboratory is immediately difficult. The relationship between different groups of compounds in brief and continuous exposure regimes is clearly an important area for further consideration. Available data suggest a wide range of outcomes may be possible from no measurable effects through to impairment of reproductive functioning and population growth.

Identification of the major toxic effects resulting from brief exposure to key classes of pesticide will enable the response of individuals to be extrapolated to a population level response. A relatively high degree of uncertainty is associated with this stage since few species have been studied in any detail. However generalised models describing rates of immigration and reproduction are of value. The data generated in mesocosm studies and other field-based systems is also extremely useful since generalisations may be made between the response of organisms in the field test system to organisms with similar life history traits in natural systems which are subject to pesticide contamination.

Headwater streams are estimated to represent more than 70% of the total length of flowing water courses in Britain (Furse *et al*., 1995). They have also been identified as significant sources of biological diversity with 20% of species identified only found to be present in headwater streams. Poorest overall quality in headwaters is associated with pastural and arable landscapes. Three principal stresses were identified by Furse *et al*.,1995 as impinging on the quality of headwater streams and these are nutrient enrichment, channel modification and drought. The results of this study provide some support for this with slurry runoff and channel clearance contributing to disturbance at one of the sites. However the presence of low levels of toxicity in stormwater was identified in the present study and it is suggested that this may result in chronic toxicological effects. Several instances of acute effects were also recorded and these are likely to be of significance since recolonisation of affected stream reaches may be very slow for some species.

With the inclusion of headwaters in future Environment Agency monitoring programmes those sites showing lowest diversity can be targeted for greater attention. The selective use of automatic sampling units and solid-phase techniques together with the identification of point source inputs in a headwater catchment will allow better characterisation of the potential contributions to toxicity. This approach together with more detailed analysis of community data for specific sites is recommended to enable the contribution of pesticides to habitat degradation in headwater streams to be more fully evaluated.

4.6 Application of techniques used in present study for future monitoring programmes

One of the objectives of the present study was to evaluate the use of different techniques for assessment of the impact of stormwater draining agricultural land, upon headwater stream communities. The bioassay procedures described in this report are modifications of standard procedures. The main point of significance is their deployment in association with solid-phase extraction methodology. The US EPA was one of the first groups to use toxicity identification and evaluation procedures incorporating a solid-phase extraction step to isolate organic compounds of medium to high polarity (Mount and Anderson-Carnahan, 1988). The technique has since been widely used to identify contributions to the toxicity or oestrogenic activity of wastewater (DiGiano *et al*. 1992, Sheahan *et al*., 1999) or process effluents (Sherry *et al*., 1997). Some recent applications have also involved the assessment and identification of toxicity in estuaries (Thomas, 1999) as well as the monitoring of pesticides in surface water (Baun and Nyholm, 1996).

In the present study the coupling of a storm event triggered sampler with subsequent solid-phase extraction and bioassay represents a powerful and relatively inexpensive tool for the

identification of toxicity in headwater streams. It is not necessary in the current approach to involve more detailed and expensive analysis to identify compounds responsible for toxicity but this option is available for selective use when bioassay results show particularly high levels of toxicity in a sample. The technique also offers the opportunity to establish the frequency of storm events above a particular toxicity threshold and in this way allows the production of toxicity information which can be used in a predictive way.

The advantages in the use of SPE with subsequent extract bioassay are:

- The technique is relatively inexpensive
- Extracted samples can be stored frozen until testing can take place
- A range of bioassay procedures can be adapted to test extract toxicity
- The potential for sub-lethal toxicity can be identified by sample concentration
- Expensive analysis to identify toxic compounds can be focused on relevant samples following bioassay.
- Large numbers of samples can be tested rapidly to provide information regarding the frequency of toxic events in streams and rivers.

The bioassay procedure using the small macrophyte *Lemna minor* which was adapted for use in this project also offers some potential for use in future monitoring programmes. The assay procedure can provide a relatively rapid indication of the presence of herbicidal activity in an extracted sample. The level of effect in the assay can also be related to longer-term effects that would result from continuous exposure to the level of herbicidal activity detected.

The evaluation of periphytic algal communities is also recommended. With general techniques such as chlorophyll or dry weight analysis together with the identification of species composition suggested for incorporation into assessments of contamination effects in headwater streams.

5 CONCLUSIONS

Headwater streams represent a significantly large percentage of the habitat available to freshwater aquatic organisms, estimated to be 62% by length (Furse et al., 1994). Pesticide applications on cracking-clay soils that are prone to bypass flow are likely to result in the occurrence of some toxic effects upon stream communities. It was estimated that following 20-30% of storm events, pesticide concentrations would be of sufficient magnitude to produce effects upon the stream fauna. An estimated 60% of storm events were predicted to result in sufficient concentrations of pesticide in the stream to produce effects upon the stream flora. The transitory nature of the peaks in pesticide concentration following storm events means that the likelihood of acute toxic effects is low but the possibility of chronic toxicity cannot be ruled out.

No obvious effects upon the drift of macroinvertebrates were recorded in this study. This may reflect the fact that natural diurnal drift patterns masked any changes due to storm events since samples could only be collected on a weekly basis. A seasonal pattern in the number and species of organism in the drift was however apparent. Some difference in the macroinvertebrate composition between sites was also present. Site D showed the most impoverished fauna of all the sites. Factors such as the proximity of a railway line, major road and nearby town undoubtedly influenced the quality at this site. Two instances of mortality of caged organisms at site D however appeared to be directly associated with the application of pesticides in adjacent fields. At site C on which hop gardens and orchards were a major land use, specific groups of organisms were absent from relatively localised areas of the stream adjacent to orchards or hop gardens.

The employment of a range of bioassay techniques together with the use of solid-phase extraction columns to concentrate pesticides present in stormwater samples allowed for increased sensitivity in the detection of low levels of toxic compounds. The generally low levels of pesticides present in samples were not sufficient to produce acute toxicity, however effects were observed at concentration factors between 1-10 times indicating that the margin of safety was low and there may be potential for chronic toxicity.

6 RECOMMENDATIONS

(i) Solid-phase extraction methodology should be more widely employed to identify the presence of sub-lethal toxicity in surface waters.

(ii) Relatively simple sampling equipment should be set up for short periods in streams draining a range of agricultural catchments to enable a more accurate estimate of the percentage of storm events that are likely to produce toxic effects in the streams.

(iii) Samples from a range of small inputs to headwater streams (e.g. silage clamps, and septic tank discharges) should be evaluated for toxicity to determine the likely zone of influence of these inputs upon headwater stream communities.

(iv) Several species representative of good biological quality (e.g. representatives of the trichoptera, ephemeroptera and plecoptera) should be tested for their sensitivity to representative pesticides from the major groups. Testing should incorporate a design that includes brief exposure to environmentally realistic levels of pesticide and also determines post exposure mortality after a fixed time.

(v) Attached communities of algae should be incorporated in headwater stream monitoring programmes.

(vi) The concentration and distribution of adjuvant chemicals in agricultural drainage samples should be evaluated. In particular the fate of nonylphenol derived from some adjuvant formulations is of particular concern.

(vii) With the implementation of controls on sheep-dip disposal, pesticide monitoring together with biological assessments are recommended for selected operations in areas with cracking-clay surface geology which is under drained, in order to refine predictions of dip mobility and potential for impact in these high risk zones.

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