SESSION 4A

ENDOCRINE DISRUPTERS – A CAUSE FOR CONCERN?

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Papers	4A-1 to 4A-4

Background evidence for environmental effects of endocrine disrupters

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ABSTRACT

This paper describes some of the main types of environmental endocrine disruption, and illustrates the issue by recounting two case histories: the oestrogenic effects of certain discharges on fish, and the androgenic effects of tributyltin on molluscs. It concludes that while more research is required to make firm predictions of the population-level consequences of endocrine disruption, and to develop effective hazard identification tests for these substances, it would be prudent to begin making voluntary regulatory controls where possible in view of their undoubted potential for environmental damage.

INTRODUCTION

Since the early 1990s, there has been growing interest in the phenomenon now known as endocrine disruption (ED). Theo Colborn, a latter-day Rachel Carson, drew popular attention to this type of pollution in a book entitled *Our Stolen Future* (Colborn *et al.*, 1996), and it is the subject of numerous research programmes, reviews, workshops and conferences (e.g. Colborn *et al.*, 1993; IEH, 1995; Toppari *et al.*, 1995; European Commission, 1996; Kavlock *et al.*, 1996; Kendall *et al*, 1998). In Britain, ED effects are now a high priority for evaluation by the Environment Agency which has recently issued a document setting out its policy for tackling the issue (EA, 1998).

ED is generally defined as any adverse change which results from chemical interference with the endocrine system, and thus embraces a multitude of mechanisms of action, including effects on growth, behaviour and reproduction. The best-known form of ED involves substances which mimic or block the action of hormones at their receptors (i.e. agonistic or antagonistic action), but it is also possible for xenobiotic substances to interfere with receptor synthesis itself, or to affect the synthesis, metabolism, transport or excretion of hormones. By far the most intensely studied type of ED concerns substances which mimic or antagonise the steroid hormones (e.g. the cyclodiene insecticide endosulfan and the pesticide formulant nonylphenol both weakly mimic the reproductive hormone17ß-oestradiol), thereby potentially producing unwanted effects on *inter alia* the vertebrate reproductive system. However, environmental examples do exist of several other possible ED effects in several taxonomic groups, including insects, molluscs, fish, reptiles, birds and mammals. Ironically, the most widely-publicised type of putative ED concerns certain effects in humans, including the declining sperm count in some populations (Swan *et al.*, 1997) and the rising incidence of testicular and breast cancer (Hakulinen *et al.*, 1986), but none of these have yet been firmly identified as being caused or potentiated by EDs (specifically, oestrogens).

It is clear that some of the effects now labelled as ED have been known for a long time (e.g. Dodds *et al.*, 1938), but the recent interest stems largely from the fact that these substances have the potential to act at often very low concentrations, and that some effects (e.g. intersex or hermaphrodite conditions) can be triggered during embryonic or larval development, but only be expressed during adulthood or in subsequent offspring.

Furthermore, particular agonists and their mimics, for example, are able to act additively at their receptors (synergistic action has not been clearly demonstrated). Many apparently unrelated but nevertheless agonistic or antagonistic substances, at individually negligible concentrations, can therefore potentially contribute to adverse effects in organisms exposed to complex mixtures. Such exposure is the norm for wildlife, particularly aquatic organisms in rivers and estuaries, and raises the possibility that current chemical-specific risk assessment procedures may be inadequate for quantifying the full range of environmental effects of new chemicals.

It is not the intention of this paper to focus solely on pesticides, ED effects having been caused by a much wider range of substances. However, readers wishing to follow up on this area can refer to Lyons (1996) who listed at least 18 pesticides as showing weak oestrogenic or anti-androgenic activity, for example. These include endosulfan (ATSDR, 1990; Soto *et al.*, 1994) which can interfere with breeding activity in fish (Douthwaite *et al.*, 1981 and 1983; Matthiessen and Logan, 1984), and *o,p'*-DDT and dicofol (oestradiol agonists) and *p,p'*-DDE (testosterone antagonist) which are responsible for a range of feminising effects in alligators from Lake Apopka in Florida (Woodward *et al.*, 1993; Guillette *et al.*, 1994, 1995 a & b).

It is also worth pointing out that although relatively little is known about endocrine systems or endocrine disruption in invertebrates, one of the best examples of ED concerns the effects of the antifoulant tributyltin (TBT) in molluscs (see below). Furthermore, much effort in the pesticide industry has been devoted to understanding and interfering with the endocrine systems of insects. In particular, several pesticides (e.g. diflubenzuron, methoprene) have been specifically designed to disrupt hormonally controlled growth and moulting processes in insect pests, and there is evidence that some of these have sometimes caused unwanted side-effects in other arthropods (crustaceans) (Cunningham, 1986). There are also reports of intersexuality in harpacticoid copepods (Moore & Stevenson, 1991) and lobsters found in contaminated estuarine and inshore waters.

The following case histories will be used to illustrate the reality of two major types of endocrine disruption in aquatic wildlife in the UK: oestrogenic effects in fish, and androgenic effects in molluscs. They draw heavily on three recent reviews by Matthiessen (1998), Matthiessen & Sumpter (1998) and Matthiessen & Gibbs (1998), where details and references can be found.

CASE HISTORY A – OESTROGENIC EFFECTS ON FISH OF SEWAGE AND INDUSTRIAL DISCHARGES

It has now been well-established that most treated sewage discharges to freshwaters in the United Kingdom and elsewhere are oestrogenic to fish (e.g. Purdom *et al.*, 1994; Folmar *et al*, 1996). In the first instance, this was detected by measuring the biomarker vitellogenin (VTG) in the blood plasma of male fish. VTG is the protein precursor of yolk and is synthesised by the liver exclusively in response to oestrogens. In males there is almost no natural oestradiol, so VTG induction in these fish is an excellent marker of exposure to exogenous oestrogenic materials. Using caged rainbow trout, Harries *et al.* (1995, 1996, 1997) have shown that some UK rivers downstream of sewage treatment works (STW) discharges are oestrogenic for several kilometres, although the effect usually declines rapidly with distance due to dilution and other processes. Furthermore, testicular growth can be retarded in the caged animals, and this effect has been replicated in laboratory experiments with alkylphenols (Jobling *et al.*, 1996). Recent results by Jobling and co-workers at Brunel University (Jobling *et al.*, 1998) have now shown that this oestrogenic exposure is accompanied in most instances by the presence of intersex conditions in wild roach. In some cases (e.g. Rivers Aire and Nene), 100% of the male fish contain oocytes in their testes (ovotestis), and effects are more marked below STW discharges in comparison with upstream stretches partially isolated by means of weirs. No UK roach populations appear to be totally free of ovotestis, but it is not known whether background levels of this condition are natural or due to the absence of completely pristine surface waters.

Closely similar effects have now been observed in wild flounder populations from some UK estuaries and coastal waters (Lye *et al.*, 1997; Allen *et al.*, 1997, 1998; Allen *et al.* unpubl.), with high levels of VTG induction and up to 17% prevalence of ovotestis in the most industrialised areas (e.g. Mersey, Tyne and Tees). Grossly malformed hermaphrodite testes and fully developed eggs in males are rare (just one or two fish with this condition have been found in the Mersey and Tyne), and no ovotestis at all has been seen in the less contaminated estuaries (e.g. Clyde, Humber and Thames). However, background levels of VTG induction have only been seen in two or three estuaries (Alde, Tamar and Dee), and even some fish caught in the central North Sea show low but statistically significant VTG induction. Possible abnormalities have also been seen in the sex ratios of North Sea dab (Lang *et al.*, 1995), and early maturation of the females of several flatfish species has been detected in various locations (Janssen *et al.*, 1997; Johnson *et al.*, 1997; Rijnsdorp and Vethaak, 1997), both effects possibly being related to contaminant exposure, although other explanations are also valid (e.g. differential fishing pressure).

Although the causes of observed effects in estuarine flounder have not yet been identified, many of the effects in rivers can be attributed to natural and synthetic oestrogenic hormones derived from conjugated material excreted by women (Desbrow *et al.*, 1996). However, other substances certainly contribute to the effects – for example, much of the response in the River Aire has been due to nonylphenol originating from wool scouring processes (Harries *et al*, 1995). The pattern of effects in flounder suggests that industrial chemicals may be contributing more significantly in that species.

The biological significance of vitellogenesis and ovotestis in male fish is now being investigated in both the freshwater and marine environments. It seems likely that there is some effect on reproductive success, but it cannot be assumed that this is resulting in population-level impacts. Nevertheless, the widespread nature of this type of endocrine disruption is clearly of concern, and probably justifies at least voluntary action to improve the quality of some discharges.

CASE HISTORY B – THE EFFECTS OF TRIBUTYLTIN (TBT) IN MOLLUSCS

The environmental effects of TBT-based antifoulants have been comprehensively described (e.g. Champ & Seligman, 1996), but the fact that these are among the best examples of endocrine disruption has only been recognised more recently (see Matthiessen & Gibbs, 1998). The first relevant observations of sexual abnormalities in molluscs were made by Blaber (1970), who observed a penis in female dogwhelk from Plymouth Sound. This effect in female neogastropod molluscs (which also involves masculinisation of the oviduct, and even sperm production at TBT concentrations above 25 ng/l) was later termed imposex, but it was not until 1981 that it was linked with TBT-based antifouling paints from marinas (Smith, 1981). Related effects now termed intersex (but without the external penis) were subsequently observed in TBT-exposed periwinkles (Matthiessen *et al.*, 1995; Bauer *et al.*, 1995), and declines in the reproductive output of oyster fisheries in the UK have also been attributed to TBT (Thain & Waldock, 1986). Worldwide, over 100 species of prosobranch molluscs are now known to be suffering from imposex (Fioroni *et al.*, 1991). This irreversible sex-change phenomenon has led to many populations being eliminated or severely depleted due to the fact that egg-laying eventually ceases when the imposed vas deferens overgrows the genital papilla, and dogwhelks do not have a planktonic larval stage to assist with recolonisation (e.g. Gibbs & Bryan, 1986). The situation in periwinkle populations is not so serious as they have planktonic larvae, but TBT has nevertheless caused some populations to have an age profile biased heavily towards old individuals (Matthiessen *et al.*, 1995). The information on TBT led to a ban in the UK and elsewhere on its use on small boats (<25 m), although the continuing uses on larger vessels are still causing impacts (e.g. Ten Hallers-Tjabbes *et al.*, 1994). To protect against these potent effects, the UK environmental quality standard for TBT in seawater is very low (2 ng/litre).

The way in which TBT causes these androgenic effects in molluscs is not fully understood, but it has been shown that female dogwhelks exposed to TBT develop high testosterone titres, and that testosterone injections can produce the full imposex syndrome (Spooner *et al.*, 1991). Molluscan steroidogenesis is similar to that in vertebrates (De Longcamp *et al.*, 1974), and it was suggested by Spooner *et al.* that imposex is caused by a build-up of testosterone due to inhibition of the mixed-function oxidase (MFO) enzyme aromatase, which in normal female vertebrates converts testosterone to oestradiol. Bettin *et al.* (1996) duplicated this work, and also showed that blocking the molluscan testosterone receptor with a suitable antagonist completely prevents imposex in TBT-exposed animals. In addition, exposure of female snails to a known steroidal aromatase inhibitor produces full imposex. This work by Bettin *et al.* and others led to the suggestion that TBT acts as a competitive inhibitor of aromatase without suppressing the MFO system itself.

While very persuasive, the aromatase inhibition hypothesis has not been proven, and it is possible that other or additional mechanisms are responsible for imposex. One possibility is that TBT prevents the excretion of testosterone and its metabolites by inhibiting sulphur conjugation or reductase activity, and limited data from periwinkles and mud snails provides some support for this (Ronis & Mason, 1996; Oberdörster *et al.*, 1998). In essence, TBT-exposed periwinkles injected with radiolabelled testosterone excrete less sulphur conjugates than unexposed ones, and field-collected mud snails with imposex contain fewer reduced testosterone metabolites. There are technical problems with some of these experiments, but the phenomenon merits further investigation.

In summary, the case of TBT and molluscs is the most well-studied example of endocrine disruption in wildlife, demonstrating a whole range of impacts from the molecular to the population level. It also shows that effects on hormone metabolism can have implications for endocrine functioning that are just as serious as receptor-mediated mechanisms.

CONCLUSIONS

It is indisputable that endocrine disruption of various types is occurring in a wide range of wildlife. The most well-known involves the effects of oestrogens and their mimics in vertebrates, but invertebrates are also affected. However, it is still too early to say with confidence that these effects are a serious threat to ecosystems. Almost the only case where widespread populations are known to be endangered is that of dogwhelks exposed to TBT, and there is consequently a need for more research on the population-level implications of other ED phenomena. There is also a need for the development of robust hazard identification tests which can be used to screen new and existing chemicals for ED action. Nevertheless, the common occurrence of effects like intersex in fish is undoubtedly an early warning which should be heeded. There is a good case to be made at this stage for voluntary action to clean up strongly endocrine disrupting discharges, and to encourage the substitution of endocrine disrupting chemicals (including pesticides such as TBT), or their use patterns, with those known to be less of a risk to the environment.

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Endocrine disruption: the evidence for mammalian effects

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ABSTRACT

A number of causal links has been established between exposure to synthetic endocrine disrupting chemicals and adverse effects on wildlife, mainly in contaminated environments. In contrast to this situation, there are currently only speculative links between endocrine effects in humans and exposure to xenobiotics. Several examples of findings in humans associated with the reproductive endocrine system have been identified, perhaps the most widely debated being the observations of reduced sperm quality. However, for there to be the establishment of an effect and causation by a xeniobiotic, certain criteria are accepted as a requirement, and these have not yet been met. The level of debate with respect to the human situation ranges from the fundamental question of whether there is a significant adverse effect present at all, through to the dissection of exposure sufficient to establish causation for a particular chemical. The elements that comprise such a debate include the study design, the reproducibility of the finding and a characterisation of the xenobiotic involved.

In toxicology in general, the characterisation of effects in animals involves agreement as to the establishment of an effect and the causation by a xenobiotic. However, in the case of endocrine disruption the variety and nature of the possible toxicological events leads to debate regarding its definition and identification in animal experiments. This is exemplified by the apparent lack of reproducibility of effects between laboratories and the debate as to whether low level effects (hormesis) present a real problem in this area, requiring greater (and as yet undefined) attention to low dose levels in experimental design. A greater understanding on both the animal and human fronts is necessary in order to ensure a balanced and appropriate assessment of the actual risk to humans.

REVIEW

Evidence for effects in humans

The possibility of chemicals causing endocrine disruption and subsequent adverse effects in the environment has captured public, scientific and regulatory attention. For this issue, perhaps more than on any single previous occasion, the toxicological concern for this perceived threat has embraced humans, and almost all of the animal kingdom. The impetus for this concern has come from two major directions. Firstly, from adverse effects demonstrated in wildlife, and linked directly to chemical exposure. These are detailed elsewhere in papers from this session. Secondly, from reported changes in the incidence of adverse health conditions in humans. These have included reports in males of increased incidences of cryptorchidism (undescended testes), hypospadias (congenital malformation of the penis), testicular cancer, a decrease in sperm quality, and in females, of an increased incidence of breast cancer (Anon, 1995; Crisp *et al.*, 1997).

These changes have been observed in medical monitoring studies and collected together as being effects on the reproductive system. In the presentation of these observations, however, there has been no link established between environmental exposure to a chemical and the production of the adverse health effects. They are, in essence, reported effects on human health looking for a cause. In view of the effects being on the reproductive system, hypotheses have been put forward whereby such changes could be rationalised as being due to endocrine disruption (Sharpe & Skakkebaek, 1993). A formal analysis of the strength of the association between exposure to chemicals and human health effects in this area has been made by Kavlock *et al.* (1996). The authors concluded that there was no clear relationship between human health effects and exposure to chemicals. The role of scientific rigour in such an analysis is necessary to allow discrimination between causality and association; this being essential if action is to be taken to reduce or remove agents found to be responsible. Consideration of the endocrine disruptor issue using the criteria put forward by Hill (1965) for effecting such a discrimination confirms the conclusions of Kavlock *et al.* and identifies that whilst the assertion is plausible, the data available do not confirm that environmental exposure to xenobiotics has resulted in adverse health effects in humans due to endocrine disruption.

One of the most discussed parameters that has been put forward as supporting a decline over time in human health, of relevance to the endocrine disruption issue, has been sperm quality in males. Many observations in this area have been made, but it was an analysis of data by Carlsen *et al.* (1992) taken from over sixty studies in several countries over a fifty year period that reported a decrease in sperm quality over time; an observation that has been used as a major part of the evidence to support the assertion of significant adverse effects in humans due to endocrine disruption. One criticism of the Carlsen paper has been that the temporal component of the data was comprised of contributions from many countries, thereby introducing a number of potential complicating factors (Fisch and Goluboff, 1996). There have been several new studies undertaken since the Carlsen analysis, following changes in the population of a single country or regional area, and whilst some report a decrease in sperm quality, others report no such effect (Swan *et al.*, 1997). Factors that have been considered in attempting to rationalise these contradictory findings, and allow an evaluation of their significance, have included methodological variations over the long (eg fifty year) time periods necessary for study; selection criteria for subjects included in the studies; and genuine regional variations reflecting etiological factors of as yet undetermined nature.

The data presently available appear to point to a reduction in sperm quality over time in some groups of individuals studied (Swan *et al.*, 1997). Further data and evaluation are required to confirm this and to investigate the extent of such changes across regions and their causes. These investigations into changes in sperm quality will need to consider environmental factors including diet, lifestyle and social activity in addition to possible chemical exposure. In the interim, the controversy in this area continues.

Evidence for effects in animals

If the current status is that there are no proven adverse health effects in humans following environmental exposure to xenobiotics, then it is legitimate to ask what the evidence is that chemicals are indeed able to cause adverse health effects in mammals through endocrine disruption. At the overview level, there is no doubt that certain materials can indeed cause such adverse effects in mammals. A number of chemicals, representing a range of chemical classes and uses, have been shown to be toxic in conventional mammalian toxicology studies (Crisp *et al.*, 1997). It is important to note that chemicals known to be active on the endocrine system in mammals include a number of pharmaceutical agents essential for the treatment of certain diseases or for hormone replacement therapy, and also certain naturally occurring materials, for example phytoestrogens such as genistein present in soya (Santel *et al.*, 1997). The effects observed in toxicity studies *via* endocrine disruption of the reproductive system may include developmental abnormalities, fertility effects and tumours in chronic studies. For these chemicals, the effects are of sufficient magnitude, and their location in tissue type, or consistency across studies, is such as to indicate an endocrine disruption origin. In many cases, mechanistic studies have subsequently confirmed and identified the nature of the lesion responsible.

The main types of effect currently under consideration in the area of endocrine disruption are effects on the estrogen, androgen and thyroid hormone systems. For such systems there are a number of ways in which chemicals can cause a perturbation, eg:

- effects on the hormone receptor
    - agonist action
    - antagonist action

- effects on hormone synthesis
    - precursor depletion
    - enzyme inhibition

- effects on hormone degradation/removal
    - enhanced clearance
    - reduced clearance

Having established that endocrine disruption can cause adverse effects in mammals, and that a variety of mechanisms for this toxicity exist, a number of studies in more recent times have focused on this issue from the mechanistic side (Holmes *et al.*, 1998), ie the beginning of the progression illustrated below:

precursor molecules ----- hormone synthesis ----- receptor binding ----- DNA transcription -----functional change ----- tissue effect (eg pathology; weight change) ----- developmental effect (eg vaginal opening) ----- tumours/fertility effect

The available conventional toxicology studies, as required for regulatory submission in support of new chemicals, represent not a mechanistic evaluation of chemicals but an apical evaluation of whether exposure to mammals at doses up to and including a maximum tolerated dose can cause adverse effects (ie from the end of the progression illustrated above). These are required as the appropriate studies on which to conduct risk assessments, in order to provide data to allow safe handling and use practices.

Alerting to potential activity *versus* defining adverse effects in animals

The battery of techniques and endpoints available for the investigation of chemicals as endocrine disruptors appears to have now moved the emphasis of studies in the literature from the identification of adverse effects in these apical animal assays to the identification of changes in any of the parameters involved in the endocrine pathways. To draw the analogy with the area of carcinogenesis, the area of endocrine disruption in toxicology has moved from evaluations in the definitive studies (cancer studies) as indicators of activity, into the area of short term-tests for predicting possible activity. We therefore now have the equivalent position to the generation of data from the bacterial mutation assays (the Ames test), the DNA binding assays, the cytogenetic assays etc, many of which are *in vitro* assays, and require an extrapolation phase to move through to possible effects in animals. All of the evidence at the present time indicates that the same level of value, but also limitations, will accompany the screens for endocrine disruption as it does the cancer screening assays (Ashby, 1997; Odum *et al.*, 1997). The availability of these assays has led to a proliferation in the number of papers reporting changes in such parameters and an increase in the number of chemicals being implicated as endocrine disruptors.

In moving down to this fine focusing, there are several requirements for the data generation that need to be satisfied for relevance to the toxicological perspective:

- the effect must be reproducible
- the effect must be due to endocrine disruption
- the relevance of the effect to the animal must be understood

These fundamental parameters are essential at this still formative stage of the derivation of testing strategies for detecting and regulating endocrine disruption. Data generated now will set the standards and thresholds for the assignment of labels and requirements for further testing of chemicals. Considering each of the three aspects listed above, there are data already available to suggest caution in each area.

- The reproducibility of data is a fundamental requisite for scientific acceptability of a conclusion. It is especially important for this area, in order to be sure of each interpretation made, since subsequent study (both mechanistic and ultimately large scale animal studies) is built on these foundations. Specifically, certain observations in the recent literature on endocrine disruption have been found not to be reproducible in other laboratories. These include the observation of decreased testis weights in animals administered benzylbutyl phthalate (Ashby *et al.*, 1997c), the observation of a lack of response in the uterotrophic assay for raloxifene (Ashby *et al.*, 1997a) and the observation of estrogenic activity for clofibric acid and benzoic acid (Ashby *et al.*, 1997b). Perhaps the most cautionary note in this area at present is the lack of ability to reproduce claims of a synergistic interaction of chemicals in an *in vitro* system, on account of the potentially enormous implications, had it been confirmed (McLachlan, 1997).

Attempts to reproduce data require careful study design themselves in order to ensure that the conclusion drawn will be valid. For example, estradiol has been reported to have an effect on prolactin levels in the Fischer 344 rat, but not in the Sprague Dawley rat (Steinmetz *et al.*, 1997). Such an effect may therefore indeed be reproducible in a particular strain of rat, but legitimate questions may then arise as to the general relevance to animals. This is a different situation however, from data that do not reproduce under identical conditions.

- Any effect seen in animal studies needs to be confirmed as due to endocrine disruption before being interpreted as such and consequent actions taken. This is particularly relevant to a number of the parameters measured in existing toxicology studies, where overt toxicity to the animal can cause similar effects to those expected from endocrine disruption. Thus, in the case of the multigeneration study, changes in the viability of foetuses/pups and their growth pattern to weaning can be caused by maternal toxicity, and similar adverse effects can be seen in developmental toxicity studies (Daston, 1994). In studies where increasingly more attention is paid to subtle changes in parameters, an understanding of the baseline level for each observation, and factors affecting them, is vital. Thus, when measuring hormone levels in mammals, the normal temporal/cyclical profile must be recognised before ascribing changes observed as due to chemicals (Bergendahl *et al.*, 1996). In the case of studies involving pups, due care must be taken in their handling and family environment to ensure no complications from an increased level of stress (Morton *et al.*, 1963).

- Changes observed in parameters relevant to mammalian systems should be considered in the context of relevance to the animal. This encompasses data generated both *in vitro* and *in vivo*. *In vitro* data may be useful for alerting to a potential for activity in the animal, but alone, are insufficient for defining such a property as relevant to mammals. This point has been recognised by the USA Endocrine Disruptor and Screening Advisory Committee (EDSTAC; Anon, 1998) in their definition of an endocrine disruptor, where activity *in vivo* is a requirement. However, for observations made *in vivo*, it is also necessary to consider the toxicological relevance to the animal. For example, the relevance and implications of a small increase in organ weight alone (eg prostate) need to be considered, rather than simply assigning a label of endocrine disruptor to a material.

Risk assessment on chemicals showing adverse effects due to endocrine disruption

The evidence therefore indicates that certain chemicals are able to cause endocrine disruption in mammals, and that under particular exposure conditions, may cause adverse health effects. Such an activity should, however, be set alongside the normal toxicological evaluations of chemicals for assessing a range of potential properties. At certain dose levels, toxicity (be it cancer, hepatotoxicity, reproductive toxicity etc) relevant to a particular chemical structure will become apparent in animals. The toxicological response can then be quantified, including the identification of a no effect level (NOEL) for the toxicity, and a risk assessment conducted to determine safe use applications. In the case of agrochemicals, current testing requirements are amongst the most rigorous for any chemical. Studies required include reproductive (multigeneration) studies, developmental (teratology) studies and lifetime (carcinogenicity) studies. It has been proposed that such study designs are sufficient for the detection of materials with significant endocrine disrupting capability (Stevens *et al.*, 1997, 1998).

One aspect to the current debate on endocrine disruption that is, however, questioning the validity of this process is the proposal that chemicals which act on the endocrine system may have a dose response that follows a bell-shape (Figure 2), rather than the conventional linear/threshold form, ie they exhibit the phenomenon of "hormesis" (Calabrese & Baldwin, 1997). With hormesis, a response is seen at a dose level below that assigned as a no-effect-level (NOEL) in a conventional toxicology study. An illustration of the most common dose-response curve showing hormesis is given in the Figure below.

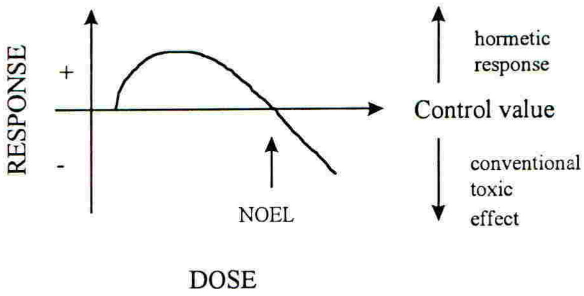

Figure. Illustration of a dose-response curve including hormesis.

The potential implications of this, if confirmed, are profound, since the possibility that current toxicological assessments may not have gone to low enough doses to identify a toxicity, would be real. The work of Vom Saal *et al.*, (1997), with reports of effects on the prostate in mice exposed to very low dose levels of chemicals *in utero*, is being taken as a test case for the existence and relevance of the phenomenon in this context. Work in a number of laboratories is currently ongoing or planned, in order to determine whether this is an issue, and if so, to characterise its extent and relevance to toxicological assessments. The USA EDSTAC committee have recommended that evaluations to assess for endocrine disruption should not allow for this at the present time, as the evidence in support of it is considered insufficient, but that a re-assessment of this position should be undertaken when the results of the above research programme are available.

In addressing the issue of endocrine disruption and its relevance to mammals, attention is currently rightly focused on two separate aspects: (i) the human epidemiological side, and (ii) the animal testing side. Further investigations into both are required in order more appropriately to define and understand the risks from chemicals that cause endocrine disruption, and any relevance to current trends in human health. Whatever the outcome, the focus of effort onto the area of endocrine disruption, both from the mechanistic and screening levels, is certain to ensure that the discipline of toxicology benefits from the derived knowledge.

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Endocrine disrupting chemicals in the aquatic environment

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ABSTRACT

The aquatic environment is the ultimate recipient for most chemicals released into the environment. This probably accounts for why much of the evidence indicative of endocrine disruption in wildlife has been obtained from aquatic organisms. Adverse effects on populations of both vertebrates (e.g. fish, reptiles) and invertebrates (e.g. molluscs) have been well documented. In some cases, the chemicals responsible for these effects have been identified, but in most cases they have not. The number of chemicals (both natural and man-made) present in the environment that possess one, or more, types of endocrine activity is already known to be quite large, and further chemicals are regularly being identified and added to this list. Many environments contain complex mixtures of the chemicals, often making it difficult to identify the chemicals actually responsible for the observed effects.

Despite the increasing evidence for endocrine disruption of wildlife, the ecological consequences of this disruption are usually not known. There have been a few well-documented cases of endocrine disruption leading to population crashes (the decimation of some mollusc populations caused by exposure to TBT used in anti-fouling paints is by far the best documented example), but in most instances the effects reported have been at the individual level, and the population-level consequences are unknown. For example, exposure to effluent from sewage-treatment works leads to intersexuality in fish, but the consequences of this to the reproductive capabilities of the affected fish remain to be established.

A number of pesticides, herbicides and fungicides have been shown to possess endocrine activity. Some of these chemicals have oestrogenic activity, whereas others have anti-androgenic activity. It is very likely that many other pesticides will be shown to possess endocrine activity; for example, our recent results show that some pyrethroids are weakly oestrogenic. Further, we have also discovered an androgenic fungicide. What now needs to be ascertained is whether or not these pesticides are ever present in the environment at concentrations high enough to cause adverse effects through their abilities to mimic (or antagonize) hormones. These recent results, and the difficulties associated with interpreting them, are discussed below.

INTRODUCTION

The aquatic environment is the ultimate sink for most chemicals, whether natural or man-made. It has been estimated that there are about 70,000 synthetic chemicals in everyday use, with another 500 to 1000 new ones being added each year. The number of natural chemicals present in the aquatic environment is unknown, but is likely to be very large. These chemicals enter the aquatic environment via many routes, including authorised discharges (particularly from sewage-treatment works) and land run-off. Some idea of the magnitude of the chemical load entering rivers can be gauged from the realisation that there are more than 70,000 consented discharges into freshwaters in England and Wales. These discharges range in volume from very small (less than $5m^3$/day) to very large (over 150,000 m^3/day); most are from sewage treatment works (STWs). Effluents from these STWs often contribute 50% of the flow of a river (especially in densely-populated areas), a figure than can rise as high as 90% (or more!) in periods of low rainfall. Thus, fish in such rivers live in diluted, treated effluent, not unadulterated water.

The chemical composition of most effluents is unknown; as each "parent" chemical will degrade into, in most cases, several or even many intermediates, each of varying persistence, which in turn will degrade further (for an interesting example, see Di Corcia *et al.*, 1998), it is clear that effluents will contain an extremely large number of chemicals. In addition, ill-defined, complex mixtures of chemicals, of both natural and man-made origin, will be washed into the aquatic environment from the surrounding land.

ENDOCRINE ACTIVE CHEMICALS IN THE AQUATIC ENVIRONMENT

The possibility that chemicals in the environment that are, or mimic, hormones may adversely affect exposed organisms - a field of research now referred to as endocrine disruption - is not new. For example, as far back as the 1930's Dodds and co-workers explored the structural basis of oestrogenic activity, and showed that a wide range of chemicals synthesized in order to very approximately "look like" the main natural oestrogen, 17β-oestradiol (such as some biphenolic chemicals and alkylphenols) possessed oestrogenic activity, albeit often very weakly so (Dodds and Lawson, 1938). Nearly sixty years later, Jobling *et al* (1995) conducted a random screen of 20 organic, man-made chemicals present in effluent, and showed that half of them possessed oestrogenic activity, when assessed in a number of *in vitro* assays, other workers have also revealed that many man-made chemicals possess weak oestrogenic activity (e.g. Soto *et al.*, 1995). Thus, it is no longer disputed that many synthetic chemicals are weakly oestrogenic (and probably also possess other endocrine activities; Sohoni and Sumpter, 1998), nor that these are present in the environment.

However, they are present together with many other oestrogenic chemicals, of both plant and animal origin (e.g. Mellanen *et al.*, 1996; Shore *et al.*, 1993), including very potent natural and synthetic "real" oestrogens such as oestradiol and ethinyl oestradiol (Stumpf *et al.*, 1996; Desbrow *et al.*, 1998).

What is much less clear is whether these oestrogenic chemicals, whether alone or in combination, are present in the environment at concentrations that cause adverse effects on aquatic organisms (see discussion below).

EFFECTS OF WILDLIFE OF ENDOCRINE DISRUPTING CHEMICALS

The possibility that chemicals in the environment that are, or mimic, hormones might adversely affect exposed organisms is also not new; for example, research published over 20 years ago (reviewed in Fry, 1995) showed very conclusively that some species of birds (particularly fish-eating ones) were adversely affected by agricultural chemicals and industrial wastes. A variety of affects on both embryos and adults was reported, including impaired differentiation of the reproductive system, eggshell thinning, and impaired incubation and chick-rearing behaviours. Often this disruption occurred at the egg/embryo stage, which is exquisitely sensitive to hormones and their mimics, but it was not until the birds matured that the consequences became noticeable.

This paper does not attempt to provide a comprehensive review of all aspects of endocrine disruption in the aquatic environment; instead, we will focus on one example only, that of "feminization" of fish in British rivers, because it illustrates our present state of knowledge, including the considerable uncertainties.

It is now nearly 20 years since grossly hermaphrodite (intersex) fish were found in the settlement lagoons of two STWs. Initial concern was focused not on the possible adverse effects of the fishery, but instead on possible effects to humans supplied with treated water abstracted from the river receiving effluents from the STWs. Research on the fisheries implications of effluent affecting sex determination, began in the 1980's. It was soon demonstrated that STW effluent was oestrogenic to fish (Purdom *et al.*, 1994). Specifically, when caged trout were maintained in effluent channels, they responded by synthesising vitellogenin (a yolk protein precursor in fish, controlled largely by oestrogens) which serves as a biomarker for exposure to "oestrogens".

Follow-up research in rivers receiving varying amounts of STW effluents showed that significant stretches of river downstream of major STWs were oestrogenic to caged fish (Harries *et al.*, 1997).

The oestrogenic potency of each river decreased with increasing distance from a STW, presumably due to dilution (and, possibly, biodegradation) of the oestrogenic chemicals. However, in the most severe case, an entire 5 km stretch of river downstream of a large STW was extremely oestrogenic - maximum vitellogenin synthesis, accompanied by reduced testes weights, occurred in the caged fish (Harries *et al.*, 1997).

Recently, the results of an extensive field study of intersexuality in one species of native freshwater fish, the roach (*Rutilis rutilis*), have been reported (Jobling *et al.*, 1998). Wild populations of roach were sampled both upstream and downstream of STWs on eight rivers and from five reference sites throughout the British Isles; the rivers selected represented a range with regard to general water quality (from very good to poor). Histological examination of the gonads revealed that a high population of the "males" were, in fact, intersex, as defined by the simultaneous presence of both male and female gonadal characteristics. Intersex fish were found at all sites, although the incidence was much higher in rivers that received STW

effluents than at the control sites; the incidence of intersexuality in "male" fish ranged from 4% (at 2 control sites) to 100% in two populations of roach living downstream of major STWs in heavily impacted rivers. There was a highly significant positive correlation between the degree of intersexuality in the "male" fish and their plasma vitellogenin concentrations (Jobling *et al.*, 1998) suggesting (but not proving) that both parameters were caused by the same factor (STW effluent).

These results provide compelling evidence that population of wild fish inhabiting many rivers in the U.K. are being exposed to oestrogenic chemicals, and that these are, in most cases, present at higher concentrations in stretches of river directly downstream from large STWs. However, the ecological (i.e. population level) significance of these results is still unclear; it is not known if the intersex fish can produce gametes (sperm and/or eggs?), whether any gametes produced can be released, and whether any gametes are viable (that is, their quality is unknown).

PESTICIDES AND ENDOCRINE DISRUPTION

It has been known for a long time that some pesticides can disrupt the endocrine system; probably the best documented example is that of DDT. Many isomers and metabolites of DDT, particularly o,p'-DDT, are oestrogenic, both *in vitro* and, more importantly, *in vivo* (Bitman and Cecil, 1970). Similarly, numerous studies have shown that methoxychlor, one of only four organochlorine pesticides still presently approved for use in the U.S., to be oestrogenic both *in vitro* and *in vivo* (Bitman and Cecil, 1970). In some "hot spots", environmental concentrations have been high enough to contribute to adverse effects which have been detected in exposed organisms, although only rarely has a direct cause-and-effect been demonstrated.

More recently, the development of rapid and sensitive *in vitro* assays for endocrine activity, particularly oestrogenic activity, has led to many insecticides, herbicides and fungicides being investigated. Quite extensive lists of active and non-active chemicals have been published (Soto *et al.*, 1995; Gascón *et al.*, 1997).

An example of the type of data that have been published recently is provided in Figure 1, which shows that methoxychlor and permethrin are both oestrogenic in a yeast-based assay for oestrogenic activity. *In vitro* assays capable of detecting other types of endocrine activity have also been developed; for example, using a yeast-based assay for androgenic activity (Sohoni and Sumpter, 1998), we found recently that the fungicide fenitrothion possessed androgenic activity (Figure 2). This is the first example of an environmental androgen eliciting a response using the human androgen receptor, to date.

The extreme sensitivity of these *in vitro* assays allows detection of very weak activity; for example, in one of the examples given below (Figure 1), methoxychlor is approximately 10,000 times less potent, and permethrin is over one million times less potent, than oestradiol; that is, these pesticides (like all others that have been reported to date to be active *in vitro*) have extremely weak endocrine activity. What this means as far as understanding whether the pesticides will show similar activities *in vivo* is very unclear presently. Very limited data are available to address this crucial issue.

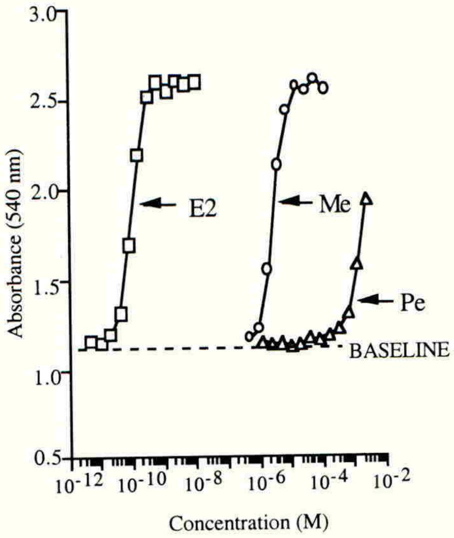

Figure 1. The pyrethroid insecticide permethrin (Pe) and the organoochlorine insecticide methoxychlor (Me) are weakly oestrogenic *in vitro*. E2 = oestradiol.

Although pesticides are some of the most thoroughly studied of chemicals, the toxicity tests presently routinely conducted were not designed to detect subtle, but possibly very important, adverse effects on the endocrine systems of the test organisms. The results of a recent, very comprehensive multi-generation study using methoxychlor (Chapin *et al.*, 1997) illustrates this point well. They reported that, when rats were orally administered admittedly very high doses of methoxychlor early in life, oestrogenic effects on the reproductive system were observed when the animals reached adulthood. There are even less data available on *in vivo* effects on fish, particularly effects (if any) on the endocrine system produced by chronic, low level exposure. As essentially all insecticides, herbicides, and fungicides will reach the aquatic environment (most developed countries have extensive survey programmes measuring the concentrations of pesticides in waterways), it is inevitable that fish, and other aquatic organisms, will receive exposure. However, this exposure is usually to "low" concentrations, although it may be simultaneously to a number of pesticides.

In summary, there is ample evidence that many insecticides, herbicides and fungicides exhibit weak endocrine activity in various *in vitro* assays. Some exhibit more than one type of endocrine activity. There is very little information available on *in vivo* endocrine effects, and

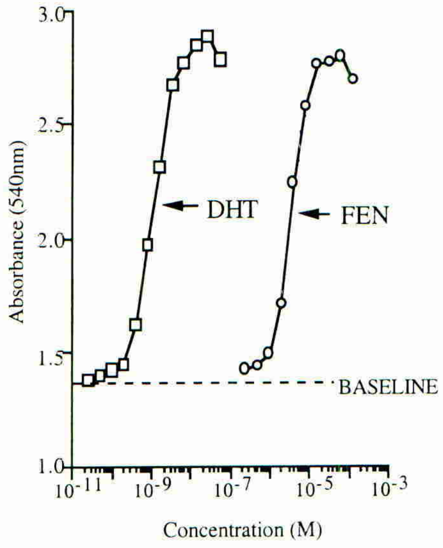

Figure 1. Androgenic activity of the insecticide fenitrothion (FEN) in an *in vitro* assay. DHT = Dihydrotestosterone.

hence it is not possible to judge whether the activities seen *in vitro* will also be seen *in vivo*. Limited data from mammalian studies suggest that, at very high doses, adverse endocrine-mediated effects, often caused by metabolites rather than parent compounds, do occur (e.g. Kelce *et al.*, 1994). No appropriately designed studies on aquatic organisms appear to have been conducted. Thus it is a very high priority to do such studies, which will involve exposure of fish to defined (i.e. measured) concentrations of pesticides, using a suite of endocrine endpoints to assess possible effects. Depending on the outcome of such studies, it may well be necessary to conduct a limited number of multi-generation studies, using a few "model" pesticides. Although such tests are very time consuming, difficult to conduct well, and very expensive, they are probably the only way of addressing (and, hopefully, answering) all of the current issues involving endocrine-active substances.

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The scientific programme of the Endochrine Modulator Steering Group (EMSG)

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ABSTRACT

The establishment of the Endocrine Modulators Steering Group (EMSG) is described and an outline given of its objectives and scientific programme. Current projects include research in three key areas: a) human health, b) environmental and wildlife health, and c) testing and testing strategies.

INTRODUCTION

The European Chemical Industry Council (CEFIC) established the Endocrine Modulators Steering Group (EMSG) in 1996 to deal with the endocrine issue and to co-ordinate major efforts within the chemical industry in Europe, particularly in research. As endocrine disruption is a global issue, CEFIC collaborates closely with other chemical industry association. The major players are the Chemical Manufacturers Association in the USA and the Japan Chemical Industry Association. Others include the Australian and Canadian associations.

CEFIC & ENSG

CEFIC is a non-profit trade association representing the larger part of the European chemical industry. The membership includes national federations from 17 different European countries, larger European producing companies and affiliated members such as the plastics producers, the chlorine industry and the crop protection industry. CEFIC does not represent the pharmaceutical or food industries.

To co-ordinate our scientific and other efforts, we work through the International Council of Chemical Associations (ICCA). It ensures a complementary programme with a minimum of overlap between the science projects of the different associations. Another organisation, which assists us in conducting our research programme, is ECETOC. This organisation is the independent scientific body of the chemical industry.

The EMSG programme is not dealing with product-related research but it is a generic programme aimed at gaining a better understanding of the issue and at developing tools for risk assessment. The EMSG three-year programme expects to spend $7 million on research.

Objectives

Research on specific products is handled by the various CEFIC Sector groups.

As an industry, we feel that we need to share the burden of public concern. The real issue is lack of knowledge and the challenge is to determine the facts. There are a large number in knowledge gaps in this area and the EMSG programme is aiming to fill a number of those and remove uncertainties.

Another objective of EMSG is that our communication with the outside world (but also within our community) is open and credible. Almost all our research is undertaken by renowned independent institutes. It is our belief that research should be unbiased with no steering by the industry.

SCIENTIFIC PROGRAMME

Our science programme focuses on three areas. They are human health and, in particular, male fertility. The leader of our industry group is Prof. Schlüter of Bayer. The second part of EMSG's science programme is developed by our environmental and wildlife health group, which is led by Prof. Randall of Zeneca. Thirdly, there is the group that is extremely active at this time in relation to the OECD process. It is led by Prof. Gelbke of BASF.

Human health

In the human health area, EMSG is advised by a Health Science Panel, made up of independent experts, observers from international organisations and industry scientists. The Panel was established in June 1997, to ensure quality of the research undertaken and a transparent decision making process. Members have recommended eight human health projects. One project is a comparative geographical study in the Nordic countries, investigating trends in sperm counts in different countries previously reported to have a big differences. The study will bring together matched populations using comparative methodology and tries to resolve whether there is a real difference in sperm counts and semen quality in those three different countries. The Nordic countries keep good records; hence, they provide the only opportunity within Europe to undertake a meaningful study. Epidemiological studies cannot be undertaken overnight, this study needs at least three years.

A similar study in the UK will encompass 6,000 men attending various infertility clinics. Again, semen quality will be investigated, and that study will also give some idea of the geographical spread and considers occupational exposures and life styles. This study is a joint project with the UK authorities and results from that study are expected within two years.

Another study supported by EMSG is carried out by Dr Weber at the Erasmus University in Rotterdam, the Netherlands. This prospective study will involve 10,000 men, their pregnant partners and their offspring. It will consider congenital malformations such as cryptorchidism and hypospadia, but also semen quality

Every study will generate extremely useful information and will contribute to help answer the question; is there really a problem with male fertility? The hypothesis is plausible but we must determine the facts.

Five other projects in the area of human health are more research-based and less descriptive. These projects will produce results within 12 months. One such project is an investigation of the possible involvement of connexins in male reproductive disorders; this is being conducted by Prof. Fénichel in France. A second project, the purpose of which is to develop an *in vitro* test for the assessment of androgenic activity (making use of prostate cell lines), is being carried out by BIBRA in the UK. Other projects are with St Bartholomew's and the Royal London Hospital, the University of Manchester and the University of Birmingham.

Environmental and wildlife health

Environmental and wildlife health is a relative new area for the industry. There is a lot of experience in environmental toxicology within the industry but the expertise in conducting field studies (e.g. on population dynamics) is weak. While for the human health programme EMSG relied on an external panel of experts, the wildlife programme was developed by EMSG's wildlife group. They identified existing peer-reviewed projects in the area of environment and wildlife health and ensured that these had been subject to thorough scientific scrutiny. Three projects emerged:

- One of the projects is by Prof. Sumpter and Prof. Karbe in Hamburg, who are collaborating to establish background levels of endocrine effects in relation to population dynamics in fish. The project is promising, but it will take time to produce results as population dynamics cannot be studied within one season. One must consider several seasons, to get some idea of the population dynamics. This is not a short-term project of the kind found in the human health programme but one lasting three to five years.

- A similar project, which has been under way for some time, is being conducted by Prof. Olson in Stockholm. His institute is studying the seal population in the Baltic Sea. EMSG is not the only sponsors of that project but we add to it in the form of, for example, a post-doctorate fellow. This allows more work to be undertaken, that was originally thought impossible.

- Another project that EMSG has decided to co-fund is the so-called EDMAR project. The majority of the funding comes from the UK Ministry of Agriculture Fisheries and Food. It includes routine environmental surveys of endocrine disruption to indicate the geographic scale of effects in vertebrates and invertebrates. The leader of this project is Dr Peter Matthiessen, from the Centre for Environment, Fisheries and Aquaculture Science (CEFAS) in the UK.

The wildlife projects are obviously long-term but they may produce some interim data.

Testing and testing strategies

The third area of our scientific programme concerns testing and testing-strategies. We in the chemical industry, have committed ourselves to the OECD process, which has established a working group dealing with the endocrine issue. It is discussing testing methods, testing strategies and assessment. The chemical industry is part of that process, and we have allocated funds to develop and validate test methods for the OECD strategy.

To give an example, EMSG is developing a protocol for a sub-chronic mammalian toxicology assay to determine which parameters can be added to make such a study a valuable tool in the first screen. Once the protocol has been agreed at OECD, we will return to the laboratory, to validate the methodology. Another method under discussion at OECD is the uterotrophic assay; again, EMSG is working on a harmonised method.
Also in the area of ecotoxicology, methods are under development and two EMSG proposals for testing have been offered to OECD. The first is to evaluate the short-term *in vivo* effects of endocrine active substances in fish and the second is to assess chronic *in vivo* effects in fish exposed to endocrine active substances.

Another important issue is investigating the value of high-throughput pre-screens (HTPS). EMSG is exploring the possibility of a collaboration with the CMA (Chemicals Manufacturers Association) from the US and the US EPA. A list of candidate substances, for testing in the HTPS, has already been identified by EMSG and the US EPA. One of the main difficulties is to find a reputable laboratory to conduct the study. Until now, only one laboratory seems to have the expertise required.

A number of other projects in the testing strategies programme are aimed at developing tools for risk assessment.

CONCLUSIONS

While the EMSG programme is substantial, we cannot meet the challenges alone. Therefore, there is a need for co-ordination of all ED research at a global level. EMSG supports the idea of developing and maintaining co-operation at all levels of expertise. This includes all stakeholders and we would like to discuss the issue of endocrine disruption in a broad forum that includes regulators and interest groups. The challenge is to bring together all the people with an interest in the subject, to make sure that we agree, for example, on international testing strategy and testing methodology. We believe that the OECD is a good vehicle for achieving that. One of our key principles is to be transparent and open as the process continues. All data will be made available through publication in peer-reviewed journals. EMSG itself will not publish the results but we will ensure that this will be done by the researchers who have been granted the EMSG contracts.

Finally, industry is willing to work together with all interested parties. Collaboration has already been established with national and international organisations such as OECD, the World Health Organisation, the European Commission and the UK authorities. Our aim is to expand our collaborations and ensure that available resources are used as efficient as possible. Only by working together on this issue we will be able to bring clarity and scientific understanding as quickly as possible to the debate.

SESSION 4B

DECISION SUPPORT SYSTEMS

Chairman	Dr J Gilmour *British Crop Protection Council, Farnham, UK*
Session Organiser	Dr D R Jones *ADAS Rosemaund, Preston Wynne, UK*
Papers	4B - 1 to 4B - 4

Decision Support System for Arable Crops (DESSAC): an integrated approach to decision support

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ABSTRACT

Advances in computer technology have enabled the development of a new generation of highly sophisticated decision support systems for use in agriculture. DESSAC will, in time, provide an integrated suite of DSS modules covering the main decision areas confronting the arable farmer. The Wheat Disease Manager module, which is nearing completion, aims to enable the user to take decisions on fungicide application based on optimising the photosynthetic capacity of the crop canopy, thus tailoring the target level of disease control to the requirements of individual crops.

WHY IS DECISION SUPPORT NECESSARY?

Farm profit must be maximised in a climate of increasing competition, falling commodity prices and increasing environmental constraints. Decisions on crop inputs have to be timely and the time of decision takers is frequently spread very thin. There is no shortage of information on which to base these decisions - rather the problem facing the decision taker is how to acquire and assimilate the wealth of data and information which are potentially available. Equally the challenge facing providers of information is how to get this information to those who need it, whether farmer or adviser. This is where computer based decision support systems (DSSs) have a potentially important role. Computers can store and manipulate large amounts of data, and DSSs enable users to focus on the critical messages from these data. If designed correctly, DSSs can also enable access to new technology which is not easily transferred in other ways.

WHY ARE DECISION SUPPORT SYSTEMS NOT WIDELY USED?

If DSSs are so useful, why are they not widely used in agriculture? There is a long history of production of agricultural decision support systems, mostly using on expert or simple rule-based systems. Whilst many of these DSSs have been used for a period, they have generally fallen fairly rapidly into disuse. A survey of UK based DSSs in 1996 listed over 20 systems, none of which had more than a handful of users (Parker, 1996a). Reasons for failure include lack of robustness, inadequate attention to the users' needs in the design phase, they were too difficult or demanding to use, they were not perceived to give sufficient benefit to the user, or the users quickly learned the key lessons so that they no longer required the DSS (C G Parker, pers. comm.). If a DSS is to be widely accepted, it must offer financial benefits over and above the existing decision mechanism through cost or time saving, or increased output.

The advent of powerful PCs, and the increasing willingness of farmers and advisers to use them, is making possible a new generation of DSSs based on mathematical models. These can simulate the development of crops and their associated pests and diseases, accounting for the impact of weather, pest pressure and other factors, and predicting the range of outcomes of possible remedial measures such as application of crop protection products. By changing the assumptions used, the decision taker can examine the likely economic impact of a range of different decisions and their associated risks to arrive at an optimum for the circumstances. It is now possible to construct such decision support tools covering all aspects of the farming enterprise. However, surveys show that a suite of independent programmes is not want users want or need. They need an integrated system which can take into account relationships between the different farm activities and share data between them (C G Parker, pers. comm.).

DESSAC

DESSAC (Decision Support System for Arable Crops) aims to meet that need. It will, in time, provide a suite of integrated computer-based decision support systems (modules) designed to address all of the key decisions facing arable crop farmers.

This will be achieved by the construction of the DESSAC 'Shell' , a generic framework (Figure 1) which will provide the software environment within which DSS modules operate and interact. The Shell will contain or provide access to data commonly needed by the modules, such as:

- climate data, recent past and forecast weather. Weather data can either be entered manually or acquired automatically from on-line weather stations or via the Internet;
- farm data such as soil type, sowing dates and fertiliser usage, so that these data need only be entered once for use in any number of modules. Alternatively, DESSAC will also be able to acquire these data from standard farm management software on the user's PC;
- pesticide data;
- data on crop varieties.

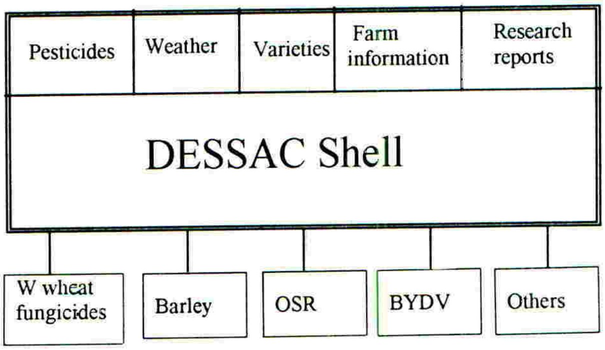

Figure 1. Simplified schema of the DESSAC Shell.

The Shell has a standard browser which will enable it to display information stored in the Shell, in the modules, or acquired via the Internet. The extensive use of hypertext links will enable the user to find rapidly the required information.

Parker (1996b) found that users require the various modules in DESSAC to have standard user interfaces with a common 'look and feel'. This is being facilitated by the construction of a 'toolkit' by the DESSAC team which is being made available to module developers. The toolkit comprises software and documentation. The software provides a set of building blocks from which developers can construct DESSAC compatible modules.

In order to 'future proof' DESSAC and to encourage module developers, as far as possible, industry standard software is being used. The basic design language is Microsoft Visual C++, communications take place through Microsoft OLE standards and the browser is derived from Microsoft Web Browser. This brings all the functionality of the standard Internet Explorer facilities such as HTML, ActiveX, and Java. Data are stored in Microsoft Access databases.

A set of criteria has been produced to ensure that all modules developed for DESSAC conform to software standards, meet well defined user needs, are based on properly validated science and that there are suitable plans for support and updating in the market.

Modules currently being developed for DESSAC include spring barley production, BYDV control, fertiliser usage and oilseed rape production in addition to winter wheat fungicide usage which is the subject of the remainder of this paper.

THE WHEAT DISEASE MANAGER MODULE OF DESSAC

The Wheat Disease Manager (WDM) module of DESSAC illustrates how DSSs can be used to transfer new technology. WDM will provide those who currently take fungicide decisions with sufficient information to permit them to make more cost-effective and timely spray applications. The need for decision support in this area is well recognised; a survey of both arable farmers and independent consultants showed that choice of pesticide active ingredient, together with rate and timing, was the most difficult of all the 'whole farm' decisions (Parker, 1995). This is particularly true for wheat fungicides which frequently represent the biggest variable cost in winter wheat production. Some 98% of the crop receives at least one fungicide spray (Hardwick *et al.*, 1997).

In order to trim costs, farmers frequently use application doses below those recommended by the manufacturers, and indeed the average rate of application on farms with more than 250ha of wheat is now only 50% of the recommended dose (J A Turner, pers. comm.). This decision is often based on very inadequate information, and care is needed to ensure that relatively small savings in input costs do not result in substantial loss of output should inadequate disease control result. The challenge is to identify those situations where cost savings are possible without incurring excessive risk, and those where greater investment in crop protection is required to achieve the crop's full potential (Paveley *et al.,* 1998a).

The scientific rationale of WDM

When susceptible wheat crops are treated with fungicides in the presence of disease, there is usually a yield benefit from treatment. Generally the greater the amount of disease controlled, the greater the benefit. However responses are sometimes greater or less than would be anticipated from a given level of disease control. Thus, if decisions on doses and timing of fungicide sprays are based purely on disease considerations, the magnitude of the yield response will be unpredictable in many instances. Yet this is the basis on which current recommendations are generally made.

It is now becoming clear that the magnitude of the response correlates better with the duration of green leaf tissue particularly after flag leaf emergence (GS39) (Tottman, 1987), than with the degree of disease control. In WDM, the crop canopy is treated as a photosynthetic unit; the aim is to protect green area which the crop cannot afford to lose to disease (particularly that which intercepts light during grain filling) but not to apply excessive inputs to protect green area that is making little contribution to yield (Paveley, in press). Decisions must be based on the likely future levels of disease, but also on the ability of the crop to tolerate that disease

Thus any individual or system which supports disease control decisions must be able to predict future disease development, and the likely response of the crop to that future disease. This requires knowledge of the various factors influencing the growth and development of the wheat crop, including disease, and how these are affected by weather and nutrition (Scott & Sylvester-Bradley, 1998; Sylvester-Bradley *et al.*, 1998; Paveley *et al.*, 1998a).

WDM will achieve this through a series of inter-related process models. These will simulate the emergence, growth and senescence of leaves forming the canopy, disease development, the effect of fungicide treatment on disease and the yield loss caused by the disease. These models are still being developed by a multidisciplinary group from Silsoe Research Institute and ADAS, using research data and information from collaborative work involving the University of Nottingham, IACR Long Ashton, Morley Research Centre, Central Science Laboratory and SAC. These models, and the research projects on which they are based, will be described in more detail elsewhere, but, briefly, the logic is as follows:

Canopy simulation

Simulation of crop canopy development is based predominantly on available nitrogen and temperature. The model predicts timing of leaf emergence and senescence concentrating on the last four leaves, and key crop developmental stages such as anthesis. Whilst the models in their current state of development are proving quite accurate when tested against experimental data, their accuracy will be improved in practice by the user checking the stage of development and canopy growth of the crop during the growing season and entering the observations using the WDM input screen. The system will use this combination of data from models and observations to quantify changes in canopy size over time, in the absence of disease.

Foliar fungal disease simulation

Models are being developed for the main foliar diseases. The risk of disease progress is calculated for the most important foliar diseases, based on disease incidence on each leaf layer (as a measure of inoculum), the resistance of the variety and the weather (past, forecast and long-term local averages).

The model for *Septoria tritici* is now well advanced. The ability of an earlier version of this model to predict the disease was described by Audsley *et al.*(1997). It has since been refined to reflect the impact of variation in the distribution and level of inoculum in the canopy, and hence the transfer efficiency from lower to upper canopy.

Models for mildew and yellow rust are also well advanced, and a model for brown rust is under development.

Fungicide action model

Eradicant and protectant dose-response curves have been determined in field trials for the major fungicides used on wheat (Paveley *et al.*, 1998b). These have been used to derive parameters describing the curves quantitatively (Paveley *et al.*, 1998a). These are used to calculate the impact of fungicide application on disease severity (and hence green leaf area index loss), based on eradicant and protectant activity and timing of application in relation to infection.

The extent to which a treatment would prevent loss of leaf area available for light interception, is then used to calculate the potential impact of the fungicide treatment on yield. Simulations using these parameters in the model give results which correspond well with field observations. Further trials are in progress to characterise newer fungicides.

Yield loss

Dry matter production is calculated for each leaf layer, based on the light intercepted by that layer, after correction for the light intercepted by the leaf layers above. The radiation use efficiency of the crop is used to calculate the dry matter produced by each layer. The partitioning of dry matter to yield depends on crop growth stage. Before anthesis, a proportion of the dry matter is assigned to yield to represent the contribution from stem reserves. After anthesis, the dry matter is all assumed to contribute to yield. The impact of foliar diseases on yield is calculated by reducing the radiation intercepted by each leaf layer according to the proportion of green leaf lost to disease.

Decision model

The process models calculate the area of the healthy leaf canopy, the likely impact of disease in reducing this green area, and the amelioration achieved by various fungicide options. The Decision Model ranks possible fungicide treatments according to their increase in margin over chemical cost. The model optimises a set of future actions (sprays) by considering their impact on the processes represented by the process models described above. It is based on genetic algorithms (Goldberg, 1989; Parsons, 1998) and is an optimisation procedure analogous to biological evolution. A randomly generated population of possible 'individuals' (spray treatments) is subject to selection based on fitness (margin over chemical cost). The process runs for several generations, and includes 'mutations' and 'crossovers' (a typical crossover would be combining the first spray from one plan with the second spray from another) to ensure that a represntative range of possible options is considered. Constraints, such as minimum interval between sprays or maximum total dose, are imposed to ensure that the output includes only treatments which fall within label recommendations. At present, the model works well for a limited range of chemicals and spray dates, and is being further developed to handle a wider range of options and to minimise calculation time.

Scenarios

An important feature of WDM is that the user will be able to ask a series of "what if" questions, for example, to examine the impact on net margin or yield of delaying or bringing forward a proposed spray application, so as to fit better with other farm operations. This can be achieved simply by changing the relevant dates and observing the changes predicted by the model. Similarly, the user could re-run the scenario using different chemicals, tank-mixes, rates or weather assumptions. Such use of scenarios would provide an insight into the robustness of various treatments, and the sensitivity to changes in assumptions. Choice of treatment can then take account of the risk the user is prepared to accept that the assumptions used by the models may turn out to be wrong; for example, the effect of future weather, which is the biggest area of uncertainty.

The final decision remains the prerogative of the user, who can combine the information provided by the system with his experience and local knowledge.

Data

The models acquire necessary data from a several sources (Table 1). DESSAC works on the principle of layering of information. For optimum performance the models require automatic (e.g. recent weather) or user input for a variety of factors, but if these are not provided the models can operate on archived data stored within DESSAC (e.g. long term climate data is used instead of actual recent weather).

Table 1. Data required by WDM models.

Data	Source
Temperature, rainfall, solar radiation etc.	Climate data from DESSAC Shell. Recent weather from local or on-farm met. station, or manual input Forecast weather from Internet or manual input
Crop nutrition, variety, sowing date	Input by user or acquired by DESSAC from farmer's standard farm management software
Varietal characteristics	From DESSAC Shell
Fungicide performance	Held within WDM
Fungicide label restrictions	Held within DESSAC Shell
Crop growth stage, disease incidence	Predicted by model and recalibrated from user observations
Grain prices, fungicide prices	Base data within WDM, but updated by user

Non-foliar diseases

Soil-borne, seed-borne and stem base diseases will be covered by an expert system which is based on the ADAS Disease Compendium (unpublished), but will be reviewed by agrochemical and independent advisers in due course.

Validation

The software components of WDM, and the science underlying them, are extremely complex, but this complexity will not be apparent to the user, whose main interaction will be via a small number of screens for inputting data, and a main screen for running the models and asking "what if" questions. The user screens (Beaulah, 1996) have been developed and refined in response to feedback provided by users in meetings and workshops during the development process (Campion & Parker, 1996). Inevitably, the main screen is quite complex because of the nature of the decisions it is portraying, but trials with potential users show that it is acceptable because it is consistent in design with other Windows™ applications and the use of the screen is easily explained (Parker, 1996b).

All decision support systems need careful validation before they are released for commercial use. This is particularly true of WDM because much of it relies on relatively new research results. A two-year programme of validation to cover usability and performance is therefore planned

The initial evaluation of usability was carried out in controlled conditions in the laboratory at the Human Sciences and Advanced Technology Research Institute (HUSAT) at Loughborough University, where the response from users was extremely encouraging. This will now be followed by two seasons of field trials with farmers and advisers, where use will be closely monitored. Answers will be sought to questions such as "is the software easy enough to use?", "can users get the answers they want and in the right format?" and "how accurate are the users' inputs to the software?". At each stage, lessons learned will be incorporated to improve the final design.

Even if the system is highly usable, it will only achieve widespread adoption if it actually improves, and is clearly seen to improve, performance. Initially, the system will be tested against data sets independent of those used in its construction. Even so, it is the efficacy in real time under a range of conditions which must be clearly demonstrated. Field trials will be carried out in the 1998-99 and 1999-2000 growing seasons across a wide range of environments, soil types and crop genotypes. Detailed assessments will measure the ability of the models to predict disease progress, crop dry matter accumulation and canopy growth, and to select optimum fungicide dose and timing. The results will be used to refine, if necessary, the parameters used in the system, and to build industry confidence in DESSAC and WDM.

Commercial development

WDM will be made commercially available by Farmplan Computer Systems for the 2000-01 crop. An organisation is being put into place to ensure proper maintenance and updating of DESSAC after its launch in the year 2000.

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Appropriate fungicide dose selection in a spring barley decision support module

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ABSTRACT

A computerised spring barley decision support module is being developed to aid selection of the most appropriate fungicides and doses for cost-effective disease control. Varietal disease resistance, nitrogen fertiliser, levels of disease and previous fungicide application are the main factors influencing dose. The module operates three approaches to disease control, prophylactic, threshold and integrated. Validation trials have demonstrated that fungicide resistance must be borne in mind when dose is determined.

INTRODUCTION

Spring barley is a short season crop. Its short life, however, means that some diseases can have an impact on growth, yield and quality if allowed to develop at most stages of growth. Hence early control of epidemics is essential. Usually, growers weigh up the risks of disease and either decide on a preventative control programme, on fungicide applications timed to control disease early in development or a hybrid between the two. Whilst prophylactic control is inefficient, it is often practised for farm management reasons or on very susceptible varieties where diseases like mildew can suddenly develop despite regular inspection. Whatever form of control programme is adopted, the appropriate fungicide dose needs to be selected at each application. A decision support module is being developed that will assist growers with this decision. This paper describes the basis of this module which will link to the DESSAC shell (DH Brooks, this volume).

FACTORS INFLUENCING DISEASE DEVELOPMENT

Varietal disease resistance is the driving force behind decisions on fungicide treatment. The risk of disease can be established from disease resistance ratings provided in the most recent recommended list. In the UK, ratings are published for mildew, Rhynchosporium, brown rust and yellow rust but not net blotch. They are determined from untreated plots of naturally infected variety trials and inoculated tests at NIAB Cambridge over three years. The data are adjusted relative to control varieties and the adjusted mean % disease converted to a resistance rating using a standard curve (Figure 1). The relationship between resistance rating and percentage disease is a direct one for ratings of 4 and above but the percentage disease is progressively greater as the resistance declines below 4. When selecting the most appropriate dose the relatively greater risk of disease at ratings of 3, 2, and 1 must be borne in mind.

Increases in nitrogen fertiliser usually result in increased susceptibility to disease. Amongst others, Jenkyn (1976) demonstrated relationships for mildew and Rhynchosporium, where there were increases in disease of 1.2% and 3.9% respectively for each 10 kg/ha nitrogen increase averaged across varieties. It is also recognised that yield optima can vary because of an interaction between nitrogen and fungicides (Jenkyn & Finney, 1981). Trials to ascertain nitrogen optima for yield and quality in the last 10-15 years have mostly received full protection against disease. Thus modifications are required to the fungicide dose if the nitrogen (including allowance for any animal manures) is significantly above or below optimum for the situation. A difference of 20 kg/ha from optimum has been adopted in the module for adjusting dose.

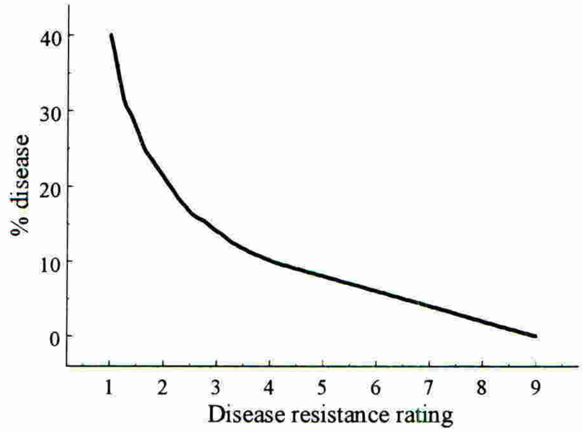

Figure. 1. Relationship between disease severity and disease resistance rating.

A series of studies in the 1980s (Wale, 1993) indicated that the threshold for optimum mildew control and yield was 75% plants or tillers fungicide showing infection. This equated to about 1% leaf area infection, which was lower than previous recommendations (Anon., 1984). Wale suggested a similar threshold for brown rust but a much lower threshold for Rhynchosporium (25%). With further studies and experience this has been reduced to 5%. A similar threshold is adopted for net blotch. Recent results on winter barley (Wale, 1998) have confirmed the relationship between incidence and severity for mildew (Figure. 2) and brown rust. The use of incidence rather than severity is less ambiguous in decision making. At the time of fungicide application, the dose required for protection, or eradication and protection, increases as the level of disease present increases.

Studies on timing of fungicides in relation to Rhynchosporium epidemics (Wale, unpublished) showed that moderate to severe epidemics usually developed when initial infection occurred before GS 30. Significant control and yield responses were achieved with applications from late tillering onwards. With susceptible varieties two spray applications were usually required. In light epidemics optimum disease control and yield response occurred from flag leaf emergence onwards (GS 37+).

When a fungicide is applied to a crop, fungicide reaches most of the surface area. Missed areas are usually protected if the fungicide has systemic action. New growth is normally unprotected (although the recently introduced strobilurin fungicides give some protection of new growth). Prior fungicide use will affect decisions on subsequent use but between the early establishment phase and GS 59 experience has indicated that for rapidly developing diseases, such as mildew on susceptible varieties, the period of protection offered may last as little as two weeks.

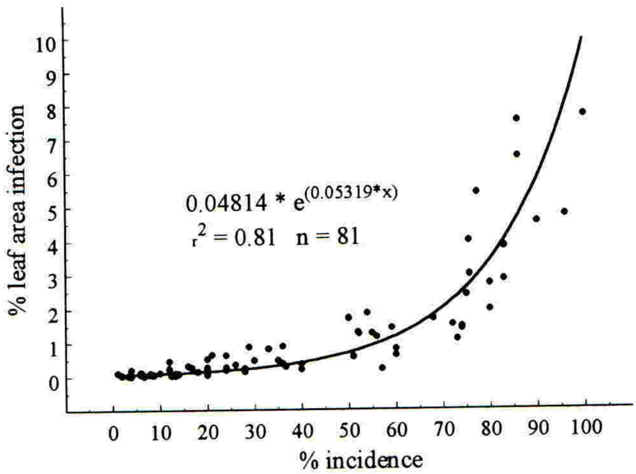

Figure 2. Relationship between incidence of mildew and severity on leaves.

Other factors influencing fungicide use are previous crop and weather. Debris from a previous barley crop may harbour the Rhynchosporium and net blotch pathogens. In consequence, previous crop is taken into account in dose selection as the inoculum pressure is higher and earlier epidemics may result. Also, the immediate past and future weather can influence epidemics. However, since the decision module is based around prophylactic or threshold disease control, weather factors are of less significance and no account is taken of them.

OPERATION OF THE DSS MODULE

The module is linked to the user's farm database. Once a spring barley field is selected and the variety identified, disease resistance ratings for that variety are used to select default doses of a broad spectrum fungicide mixture from a matrix. This default dose is considered the appropriate dose to control those diseases the variety is most susceptible to if applied at the thresholds indicated above, and is thus related to the disease risk. For example, in the version of the module currently being evaluated, using a mixture of fenpropimorph (Corbel, 750 g a.i./litre) and flusilazole (Sanction, 400 g a.i./litre), the default for the variety Prisma (resistance ratings for mildew, Rhynchosporium, brown and yellow rust of 3, 6, 6, 8 respectively) is 0.35 litres/ha Corbel + 0.15 litres/ha Sanction.

The matrix of default doses have been determined from annual SAC spring barley fungicide trials from the mid-1980s onwards and from appropriate fungicide dose studies (Wale, 1998), including those evaluating interaction of host resistance and fungicide dose (Figure. 3). Early studies (Wale, 1993) indicated that for mildew, even on a highly mildew susceptible variety like Golden Promise, quarter doses of a triazole/morpholine mixture were sufficient for effective control. More recently, trials have suggested that these doses are too low, illustrating that development of resistance of pathogens to fungicides can influence the effectiveness of a disease decision support system. Regular re-evaluation and updating are essential.

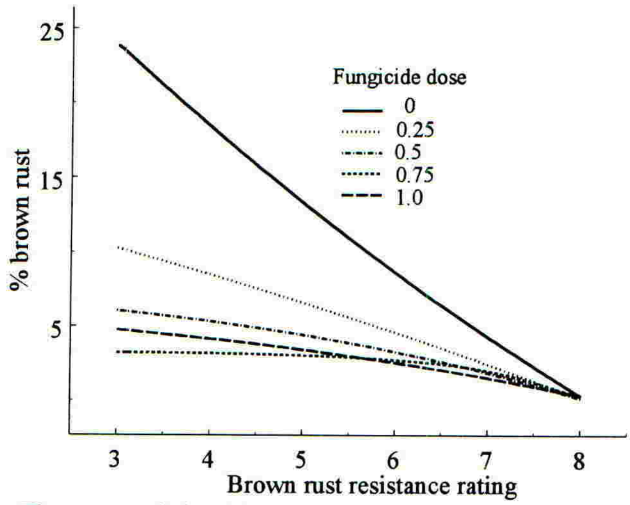

Figure 3. Relationship between fungicide dose response and host resistance to brown rust.

The user first defines the approach to disease control, Threshold, Prophylactic or Integrated. The Threshold option depends on, at least, weekly crop monitoring and recommends a fungicide application when any disease reaches its threshold. The default dose is applied once the threshold is reached but the dose is increased if disease is greater than threshold. No fungicide is recommended before GS 12 because of limited ground cover. If early mildew develops at this stage a low dose, low cost eradicative treatment is recommended at GS 13. From GS 13 to GS 59 fungicide applications are triggered when diseases reach thresholds, the default dose being adjusted up or down depending on deviation from optimum nitrogen or where thresholds are exceeded. Between GS 59 and GS 69 if a disease threshold is exceeded, fungicide is only recommended if yield potential > 6 t/ha and the crop has a high value (e.g. for malting). At any growth stage, if a fungicide was applied in the previous two weeks no fungicide is recommended. After GS 71 no fungicide is recommended irrespective of disease levels.

If the Prophylactic option is selected, a default programme of fungicides is constructed based on the disease resistance ratings. For example, this is a three spray programme for the cv. Prisma. Every opportunity is taken to apply fungicides when other applications are planned. Thus if a herbicide is to be applied between GS 13 and GS 15, the first fungicide of the programme is included at this time unless disease thresholds are reached earlier. Subsequent fungicide applications are planned at 3-4 week intervals but intervals reduced if monitoring indicates thresholds are achieved earlier. The default fungicide doses are selected as before but also take into account whether the previous crop was barley. Effect of growth stage on fungicide application follows the same pattern as for the Threshold approach. The Integrated option attempts to apply fungicide with other applications but treatment is based on risk assessment along with disease thresholds.

Selection of equivalent fungicide doses

The Winter Barley Appropriate Fungicide dose project (Wale 1998) has provided comparative fungicide dose response curves for a range of popular barley fungicides for each of four foliar diseases. Both protectant and curative situations were evaluated. Typical curves for Rhynchosporium in a curative situation are shown in Figure. 4. Using these data, comparable doses can be determined to present the user with a range of control options in terms of efficacy or cost effectiveness.

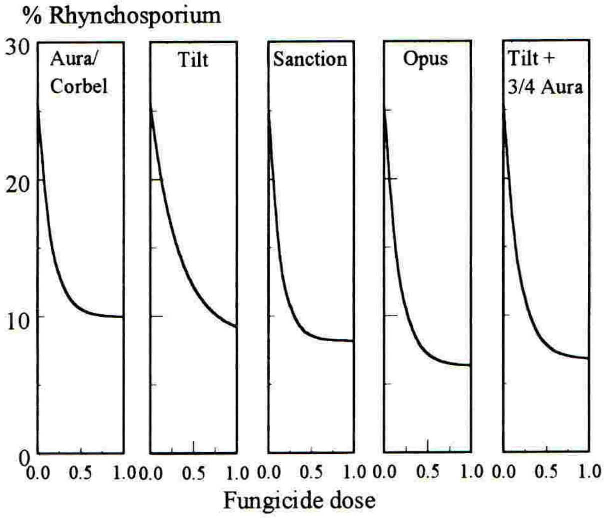

Figure 4. Curative activity of fungicides against Rhynchosporium

Fungicide mixtures

Trials evidence has shown that a triazole/morpholine mixture is more effective against mildew than morpholines alone (Wale, 1993). The principle reason for a mixture, however, is that fungicides from unrelated groups showing no cross resistance minimise the build up of insensitivity to fungicides (Anon., 1996). Experimental studies (e.g. Bolton & Smith, 1988) have supported this view. Thus fungicide options presented in the module are all mixtures of fungicide from different groups.

VALIDATION OF DSS MODULE

A number of trials have been initiated in 1998 to validate the module. In each trial, Prophylactic, Threshold and Integrated fungicide programmes determined from the model are evaluated against a maximal programme. Additionally, the dose indicated for the Threshold approach is compared to doses less and more than indicated. Data from one trial are shown in Table 1. For the Threshold programme, the first application was made when mildew was present on just over 75% plants. There were no significant differences in disease control between the three programmes and the maximum dose when using the same fungicide. However, the combination of Ensign (containing a strobilurin) and Sanction resulted in significantly better disease control. It is surprising that the high doses of the triazole/morpholine mixture in the maximum treatment did not achieve near complete control. Recent studies (F. Burnett, pers. comm.) have indicated that mildew sensitivity to morpholines has declined in recent years, supporting the increase in dose levels required for effective control since the 1980s. Yield and grain quality results are required before refinement of fungicide doses is made.

Table 1. Spring barley decision support validation trial on cv. Prisma, Aberdeenshire, 1998.

Treatment	Fungicide combination	Total dose (2 sprays)	Mildew (% area infected) 9 July 1998 Leaf 2	Leaf 3
Untreated	-	-	13.7	19.7
Prophylactic	Corbel + Sanction	0.8 + 0.35	6.5	4.6
Integrated	Corbel + Sanction	0.8 + 0.35	7.3	5.3
Threshold	Corbel + Sanction	0.8 + 0.3	7.2	6.4
Threshold -	Corbel + Sanction	0.7 + 0.2	5.8	6.0
Threshold +	Corbel + Sanction	0.9 + 0.4	4.4	6.1
Maximum	Corbel + Sanction	1.5 + 0.8	4.0	3.8
Threshold	Ensign*+ Sanction	0.7 + 0.3	0.2	0.1
LSD ($p<0.05$)			2.18	3.06

*Ensign = 300:150 g a.i./litre fenpropimorph:kresoxim-methyl

-/+ indicates a reduction or increase in threshold dose

ACKNOWLEDGEMENTS

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MORPH: Expediting the production and distribution of decision support systems to the horticultural industry

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ABSTRACT

Personal computers have long had sufficient power to run mathematical models describing complex biological processes. A number of mathematical models of horticultural pests and diseases have been developed into computer models by Horticulture Research International for use by growers. These decision support systems can be developed a stage further, to increase their 'ease of use' and decrease their 'cost of ownership' by using common protocols such as MORPH. In this way taking advantage of developments in fully automatic weather stations and the Internet.

INTRODUCTION

Over the last 10 years, Horticulture Research International has developed a number of mathematical models of various pests and diseases that effect the horticultural industry. With additional help from various funding bodies such as the Horticultural Development Council (HDC), the Apple and Pear Research Council (APRC) and MAFF these models were taken forward to computer decision support systems. These computer decision support systems were made available to the industry. Examples are computer systems such as ADEM, PESTMAN, WELL_N, BROCCOLI and SPACING.

The market penetration of these systems has been initially disappointing. The reason for the poor take-up rate could be:

- Grower's perception that no benefit is gained through using these decision support systems.

- Grower's perception that the benefit gained is not worth the time, effort and expense of using the decision support systems.

- Growers not aware of the decision support systems.

The financial benefit provided by these models is not in doubt. In research, growers themselves estimated the theoretical benefit gained through the use of decision support systems to be as much as £2000 per ha per annum (Lucey *et al.*, 1997).

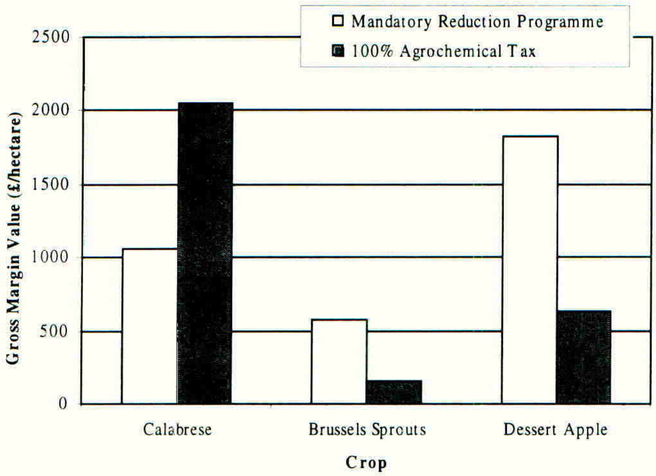

Figure 1. Benefits of using Predictive Models.

The above figures (Figure 1) were calculated by growers and advisers themselves in workshops held by Horticulture Research International. In the workshops, the growers were asked to calculate the financial implications of the increasing pressure to reduce agrochemical input in horticulture. Two specific scenarios were considered:

- A 100% agrochemical tax imposed over two years.
- A mandatory agrochemical reduction programme achieving a 50% reduction within two years.

The figures represent the mean average values reached by all the workshops. One of the assumptions made was that the results from one weather station were applicable to 100 hectares of vegetables and 20 hectares of fruit. These values were suggested by a weather station manufacturer, however they are debatable. The subsequent sensitivity analysis suggested that a single weather station needs to cover around 10 hectares to remain beneficial.

However, despite the favourable financial assessments of decision support systems given by growers in workshops, the take up of models like these was disappointing. This suggested that the systems required too much of the growers' resources (time and money) to use.

Horticulture Research International in discussion with the industry recognised the following areas of decision support systems required improvement:

- Weather data handling
- Information sharing between systems.
- Displaying results to the user.

The main input to these decision support systems was weather information. The pest models tended to be driven by temperature. The disease models tended to be driven by temperature and wetness (rainfall, leaf wetness or both). In the past, the cost of capturing of this weather information was prohibitive to the use of these computer models. The weather information tended to be gathered from a local weather station installed in the field. In the past the weather stations fell into two camps:-

Expensive automatic weather stations with automatic data transfer via modem or radio communication. However, this automatic communication brings the weather data into a propriety format. A further stage is often required to transfer the data into a format the models can use. This amount of effort on the part of the grower and the cost of the station discourages use of computer models.

Less expensive weather stations tend to require a visit to transfer the data onto a portable computer. This takes a great deal of time and discourages use of computer models, especially at critical times of the season.

A further limiting factor on the use of a large number of decision support systems was their disjoint nature. The situation was that having spent time transferring weather and other data into one decision support system, the same weather and other data needed to be transferred / entered into subsequent decision support systems. These subsequent decision support systems did not refer to other decision support systems in use. This was a complete waste of the growers valuable time. Related to the disjoint nature of the decision support systems was the fact that each system had a different approach to data entry and displaying results. This resulted in the grower having to learn a new skill set for each decision support system.

Horticulture Research International set about producing a protocol and software to address these issues. The software was given the name MORPH, which is derived from models of research practice in horticulture.

METHODS AND MATERIALS

During 1995, work began on the MORPH decision support system. The principal idea was that MORPH would be a modular system, with each module replaceable in the future as technology changed.

The system was designed to provide a common standard for weather data. This standard would enable all decision support systems to use a single weather database. This would reduce the amount of effort required to get weather information to the decision support systems. Once the weather information is stored in the MORPH database, all the decision support systems are able to access the data. This also enables all MORPH decision support systems to work with a comprehensive range of weather stations. Previously each decision support system was loosely tied to a particular weather station. MORPH would also enable new weather gathering techniques such as weather networks and weather data from the Internet to be added at a later date and be backwards compatible with existing decision support systems.

The MORPH system was also designed to contain a single database of additional information, which is made available to all decision support systems. This was intended to reduce the amount of time spent by the grower entering information. The grower would not need to enter the same information twice, since additional decision support systems could access the database rather than require the grower to supply the information again. Also, once the grower was familiar with entering data for one decision support system, he became familiar with the data entry of all MORPH decision support systems.

Another feature was a common output protocol. This was designed to allow new report viewer programs to be developed, as different output types became required. The initial and default report viewer is a straightforward on screen view of tables and graphs. This viewer can be used to zoom into critical areas of graphs and print output to Windows supported printers etc. Soon after MORPH was first used a requirement for an html output arose. This was quickly developed with the result that all the MORPH decision support systems were able to produce reports in html format to put straight onto a web server.

RESULTS

MORPH was first used for the 1997 season. Valuable lessons and improvements to the system were gained through use by growers. The common theme throughout all the comments and suggestions made regarding MORPH was that the users wanted to spend less time actually using the system before results were obtained.

The activity that occupied most amount of time was transferring weather data into the system. At this time, the majority of users had weather stations that transferred data via a floppy disk. This meant that the user had to physically visit the station with a portable computer and download the weather data. Then when the user returned to the base computer the weather information had to be uploaded into MORPH. This resulted in the user needing to spend a lot of time updating the weather data and the system being behind the current time.

For the current season the cost of direct communication with weather stations via either cellular telephone or radio has dropped. The majority of users have decided to upgrade their weather stations along these lines. This means that MORPH can talk directly to the weather stations to get the up-to-date weather information without the user having to leave the desk. In practice, with the met station drivers we have currently, MORPH tends to talk to the weather station's own software rather than directly to the weather station.

Another requirement was for a more permanent record of the decision support systems reports. This was facilitated by various printouts that the decision support systems supplied. However, during the course of the season a new feature to view the decision support systems report without running the system was added to MORPH. This means that each result obtained on previous runs of the decision support system is made available via the main menu. This enabled users who were not skilled in the use of the decision support system to view the reports without interacting with the decision support system in any way.

For the 1998 season, two new major features were added.

The first is a scheduler within MORPH itself. This allows MORPH to create decision support system reports and download weather data automatically at pre-appointed times. While this was a relatively small change in MORPH itself, it was a more profound change to the decision support systems and weather station drivers. The way a decision support system or a weather station driver responds to an error when it has been activated automatically has to be different to the way an interactive system responds. Displaying a user prompt and waiting for the user to click OK is not acceptable. The scheduler is most powerful when used in conjunction with an automatic weather station. This allows MORPH to obtain up-to-date weather information and supply the user with new decision support system reports each morning or week.

The second addition is a short-term future weather generator. In addition to the current and past weather data obtained from weather stations, MORPH makes available long term average and typical weather data. It was always intended to allow future short term weather forecasts to be entered into MORPH. The initial design was a graphical display of weather variables, where the user would be able to drag the graphs around to simulate future weather. This is still our intention. However, a simpler first step is to allow the decision support systems to generate some future weather that will produce interesting results. In practice, this means that decision support systems can generate future weather similar to the current weather but with critical weather conditions modified. For example, those decision support systems concerned with diseases can generate future weather similar to the current conditions but wetter or drier; those concerned with pests can generate future weather similar to the current but warmer or cooler. Since the limiting factor on diseases tends to be wetness and on pests it tends to be temperature, these types of scenarios tend to produce the most significant changes. The growers very often ask for the worst case weather scenarios, which this system hopefully can give them.

CONCLUSIONS

Growers do believe that decision support systems provide targeted information, which financially enhances their business. More growers have more computer power available to them. Growers are prepared to buy hardware (weather stations) to use decision support systems they regard as useful.

The less time a grower has to spend using a decision support system before it supplies a result the more attractive and widely used the system will be. The growers have a strong desire for ever greater automation in these systems.

MORPH provides an essential framework to adapt rapidly to these continuing improvements in technology.

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An Internet-based decision support system for the rational management of oilseed rape invertebrate pests

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ABSTRACT

Invertebrate pests are serious threats for growers of oilseed rape. Traditionally, management of one pest has been done in isolation of any other. However, the control tactics used for one may impinge upon the management of other invertebrates. A decision support system, named MOPI, has been developed to manage the invertebrate pest complex of oilseed rape holistically. At present the system contains procedures for the concurrent management of five pests. An Internet version of the system has been developed to facilitate the delivery of information.

INTRODUCTION

Oilseed rape (*Brassica napus*) is under threat throughout its growing season from invertebrate pests. Of the complex of pests that attack the crop, eight have been identified as being of major concern (Figure 1). Pests that attack in the winter are cabbage stem flea beetle (CSFB; *Psylliodes chrysocephala*), rape winter stem weevil (RWSW; *Ceutorhynchus picitarsis*), slugs and aphids as virus vectors (A/V), while the main summer pests are pollen beetles (PB; *Meligethes* spp.), cabbage seed weevil (CSW; *Ceutorhynchus assimilis*), brassica pod midge (BPM; *Dasineura brassicae*) and cabbage aphid (CA; *Brevicoryne brassicae*). Historically, decisions on appropriate control have been made for each pest individually without consideration of the consequences for the management of any other pest. However, managing pest outbreaks individually could lead to unnecessary application of pesticides and a more rational approach would take into account the pest complex as a whole rather than concentrating on particular pest problems.

Holistic management decisions relating to a pest complex are difficult to make empirically because of the complicated interactions between the pests and appropriate control tactics. Computerised forecasting and decision support systems (DSS) have been developed for individual pests on other crops but few have been produced which generate simultaneous recommendations for more than one pest at a time (Morgan & Solomon, 1993). The current work will describe the development of a computerised DSS for the multi-concurrent management of the major pests of oilseed rape.

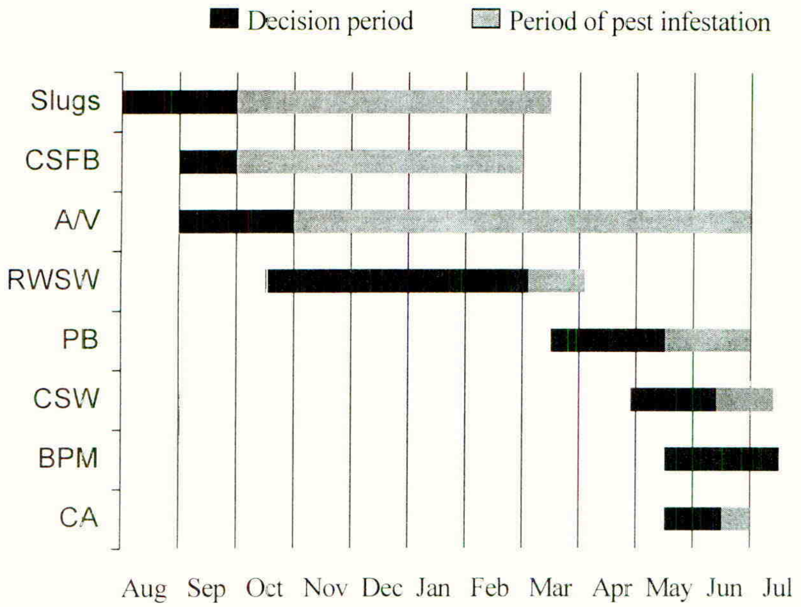

Figure 1. Potential period of damage from major pests of oilseed rape and time of sampling for control decisions; CSFB = cabbage stem flea beetle, A/V = aphids as virus vectors, RWSW = rape winter stem weevil, PB = pollen beetles, CSW = cabbage seed weevil, BPM =- brassica pod midge, CA = cabbage aphid (after Walters & Lane, 1994).

METHODS AND MATERIALS

Pest models

At present concurrent models have been developed for five pests; CSFB, CSW, BPM, PB and CA.

CSFB is a widespread pest of oilseed rape throughout the UK. Both adult and larval stages feed on plants causing extensive damage. Decisions on whether pesticide applications are warranted are dependent upon an assessment taken within the crop. Originally, 20 plants were taken randomly within a field and the leaves and stems dissected to determine the number of larvae present; if population densities of five or more larvae were recorded then treatments were advised, whereas if populations were lower then five then no treatment was recommended (Lane & Walters, 1993). However, the dissection of plants has proven costly, laborious and time consuming and an alternative sampling method was developed whereby externally visible symptoms of larval tunnelling ('scars') were used as estimates of pest infestations and hence basis for treatment recommendations (Cooper & Lane, 1991).

Larval stages of CSW cause damage to oilseed rape crops by eating developing seeds within the pods but insecticide treatments are aimed at controlling the adult weevils before they lay eggs. Determining the need for sprays is dependent upon assessing adult weevil numbers within the crop; 20 plants were beaten gently so that the deposed insects were caught in a shallow tray from which the number of adults weevils were recorded (Cooper & Lane, 1991; Lane & Walters, 1993). However, this technique was inconsistent, and was improved by incorporating a temperature-mediated behavioural component whereby the numbers of weevils caught from beating the crop was corrected dependent upon the ambient temperature when the sample took place (Walters & Lane, 1994).

BPM is a common pest of winter oilseed rape which can cause severe damage in occasional fields. Females utilise the punctures left by feeding CSW to lay their eggs within developing pods. The resulting larvae feed gregariously within the pods such that pods can shatter prematurely resulting in significant seed loss. Decisions on the need to spray against BPM utilise the link between CSW feeding punctures and BPM egg laying whereby the control of adult weevils confers management of the midges. However, as BPM outbreaks tend to be localised, management decisions are based on a history of midge and lower treatment thresholds for CSW (Lane & Walters, 1993).

PB warrant management only on backward/poorly grown winter oilseed rape crops and are only a threat if present in very large numbers during the susceptible green/yellow bud crop growth stages. A similar decision process to that for CSW is used to manage PB; 20 plants are beaten into a tray and the number of beetles caught recorded. In well grown crops, if the number of beetle sampled exceeds fifteen per plant, treatment is recommended, while in backward or poorly grown crops the treatment threshold is reduced to five (Lane & Walters, 1993).

Large infestations of CA occur on winter oilseed rape on flowering and post-flowering crop growth stages, but such damaging outbreaks tend to be very sporadic. A simple presence/absence assessment method is used to determine whether pesticides are justified; if greater than 10% of the racemes in a field are infested with aphids then treatment is recommended but if infestations are below this threshold then sprays are not justified (Ellis, *et al.*, 1995).

DSS structure

A schematic diagram of the DSS, named MOPI (Management of Oilseed rape Pests via the Internet), is given in Figure 2. The structure of the system can be divided into two interdependent components: decision-making models and supporting information.

MOPI has been developed for use via the Internet. It has been produced using the programming language Java (Microsoft Inc.) and the Internet database management system Cold Fusion (Allaire Corp.) and is implemented on a Sun 20 server (Sun Microsystems

Inc.). Use of standard Internet browser techniques, for example hyperlinks, have been fully utilised within the DSS to facilitate ease-of-use. Particular attention has been given to security so that data maintained within MOPI can be accessed only by the appropriate users; farmers and consultants can only view and use the information that relates to their pest outbreaks.

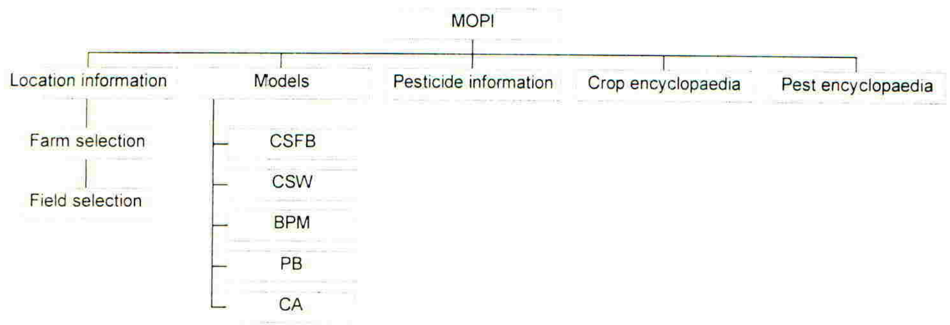

Figure 2. Schematic diagram of MOPI structure (pest abbreviations as in Figure 1).

The first database within MOPI used is the location database. On entering the system, users are required to select their farm and specific field on the farm for which they want recommendations. Thus MOPI was developed to produce advice for individual fields to improve the precision of pesticide advice by recommending applications only where and when necessary. The location database is secure so that users can only access information regarding the farms and fields they have already configured.

Following selection of appropriate farms and fields, users can implement the models by clicking on a picture or the name of the pest. Models have been developed to be operated concurrently whereby the actions recommended by a specific model can alter the potential advice generated from another model. Furthermore, the models have been structured so that minimal input is required from the users (a paramount design feature realised from interaction with potential end-users) and so that results are presented in an easy to understand yet informative format. Users can also interrogate the models to determine how the results were derived by examining logic flow diagrams contained with the system. Another dynamic feature of the models is that they allow users to change their input interactively so that they can to derive a series of results and advice and review them comparatively.

If a model recommends that pest levels warrant chemical intervention, then users are directed to the pesticide database. To date, the database contains information on 53 insecticide products consisting of 26 different chemicals that are recommended for control of all major and minor pests on oilseed rape. Of the five major invertebrate pests within

MOPI the database contains 35 insecticide products recommended against cabbage stem flea beetle, 16 against pollen beetles, 6 against brassica pod midge, 4 against cabbage seed weevil and 25 against cabbage aphids.

Throughout MOPI there are facilities to retrieve encyclopaedic information relating to oilseed rape crops and its invertebrate pests. These facilities provide background material to aid users make more informed decisions.

RESULTS

Pest models

The thresholds used in all models have been validated thoroughly against field results of pest incidence. Data from the CSL/ADAS surveys of pests and diseases of arable crops from 1990 to 1995 were compared with the model predictions. Records from over 300 fields collected over the five years indicated a significant relationship between model predictions and field results.

DSS structure

A prototype version of MOPI has been developed and implemented over the CSL Intranet to facilitate testing of the software. The system has proven a useful tool in pest management not only as a mechanism to deliver advice and recommendations but also as an information/education tool. Responses from users have been positive and, although the use of Internet technologies are relatively new, most users have familiarised themselves quickly with the system.

DISCUSSION

Decision models for five out of the eight major invertebrate pests of oilseed rape have been integrated. Validation of the models at many sites throughout the UK over several years have proven successful and hence have been integrated into a computerised DSS.

MOPI has several unique features to aid users derive the information they require quickly and easily. Not least is the system's ability to allows users to experiment with their input data to get generate a series of potential pest scenarios and review them comparatively. This was felt to be an important feature of the system as it not only allows decision-makers to experiment with management options directly but also highlights and raises their awareness of the relative importance of various aspects of rational pest management (Morgan, *et al.*, 1997).

Furthermore MOPI has been developed with leading-edge Internet technologies to improve its delivery to end-users. Much has been made of the use of the Internet and the World Wide Web (WWW) as a mean of disseminating information and there are negative as well positive aspects. For example, concern has been voiced at the lack of sufficient security within Internet-based system and that sensitive information can be readily extracted from them. However, with recent advances with on-line banking and financial transactions, Internet security is much improved and MOPI utilises the same technologies to ensure that users can only view/utilise their own information.

One of the advantages of Internet-based systems is that only a single version of the software is implemented on a server computer at any one time and users have to access the program remotely from their own computers. Since there is only one copy of the DSS running, any changes, updates or 'bug' corrections to the software have to be made only to a single program and can be implemented quickly and cheaply, thereby negating the cost and inconvenience of revising the system, copying the changes onto suitable media and distributing it to customers.

DSSs can be beneficial in the derivation of rational pest management strategies. They can assist users by synthesising complex information/data/results into understandable formats which will help decision-makers execute more informed and rational management schemes. Furthermore, their role is likely to become more important as practical and prudent agriculture becomes ever more complex.

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POSTER SESSION 4C

FATE AND EFFECTS OF PESTICIDES IN THE ENVIRONMENT

Session Organisers Dr I Barber
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Poster Papers 4C-1 to 4C-5

The prediction of the fate and effects of pesticides in the environment using tiered laboratory soil microcosms

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ABSTRACT

Most studies reported in the literature, which assess the effects of pesticides on soil organisms and processes, and their fate and leaching, are related to one species of organism, one soil process or one fate aspect of the investigated pesticide. These studies are usually performed according to standard test guidelines recommended by competent national or international authorities and legislation responsible for the registration of pesticides.

Over the last three years, in an international collaborative program, we have been discussing the use of terrestrial microcosms as model ecosystems to assess simultaneously the overall effects of single pesticides on a range of soil organisms, as well as soil processes.

In this paper we introduce two different kinds of terrestrial microcosms which can be used in a tiered testing approach. Firstly, small replicated laboratory **integrated soil microcosms** (ISM), containing sieved soil, introduced and indigenous invertebrates and a single plant species. Secondly, larger **terrestrial model ecosystems** (TME) consisting of intact soil cores from the field maintained under laboratory conditions, with less replication, but containing a greater diversity of indigenous invertebrates and mixed plant flora.

INTRODUCTION

The maintenance of soil quality, fertility and structure is essential to the protection and maintenance of the biodiversity of terrestrial ecosystems. To protect such systems, a range of carefully designed studies to assess the effects of pesticides on key species of soil organisms, and on particular soil processes, have been developed by national pesticide registration authorities, testing organizations and international communities such as the European Union. Most of these tests are used to assess the impact of individual pesticides on specific groups of soil organisms such as: bacteria, fungi, protozoa, mycoplasma, springtails, earthworms and beneficial arthropods, in the laboratory (European Community, 1991). Individual tests have also been used to assess the effects of pesticides on specific dynamic soil processes such as organic matter breakdown, soil respiration, enzyme activity and nitrogen transformations (Römbke *et al*., 1996) as well as the degradation pathways, leaching and environmental fate of pesticides.

These predictive toxicity tests are usually conducted in the laboratory and do not account for interactions between organisms or populations and their environment. The objective of assessing environmental effects, however, is to determine whether the integrity of the ecosystem is perturbed when pesticides are introduced into the system. Since integrity is a function of the structure and function of an ecosystem, the impacts of chemicals in the environment should be assessed for both structural and functional effects.

In recent years, the potential of using laboratory or semifield terrestrial microcosms to bridge the gap between single-species toxicity tests and the field to assess the effects of pesticides on soil ecosystems has been explored (van Voris, 1985; ASTM, 1988; Römbke *et al*., 1996; Sheppard, 1997). However, although these microcosm studies measured several endpoints, they did not fully integrate the data or assess the full environmental potential of such data for predicting environmental impacts. The concept has more recently been extended by our group into the design of integrated soil microcosms or model terrestrial ecosystems that involve a wide range of endpoints. Details of these have been reviewed and discussed by Knacker & Morgan (1994), Morgan & Knacker (1994), Edwards *et al.* (1994), Edwards *et al.* (1996) and Bogomolov *et al.* 1996).

In this paper we introduce two different kinds of soil microcosms which can be used in a tiered testing approach, with effects of a pesticide at one testing level triggering tests at the next level. The proposed tiered levels are:

- Small, **integrated soil microcosms** (ISM) with sieved soil, selected introduced invertebrates, single plant species to measure effects of pesticides - highly replicated environmentally-controlled laboratory studies.
- Larger, more complex, integrated **terrestrial model ecosystems** (TME) with intact soil cores, containing indigenous microorganisms and invertebrates, greater biodiversity, mixed plant flora to measure effects of pesticides - less replicated, laboratory semi-field studies.

Furthermore, we present basic methodological aspects of the ISM and TME and describe the measurement end-points as well as the design of preliminary tests conducted with ISM and TME using carbendazim as a model pesticide. Results will be presented elsewhere and will be used to decide on the final test design for the microcosms and the field validation study to be conducted.

METHODS

Integrated Soil Microcosm (ISM)

The microcosm consists of well-defined, thoroughly-mixed, sieved, field-collected, soil containing endogenous microorganisms, microarthropods and nematodes, packed gently into a plastic cylinder, with wheat seedlings planted in the soil (Edwards *et al*., 996). Each microcosm is 7.5 cm inside diameter x 15 cm high, constructed from commercially-available high density polyethylene (HDPE) pipe. Fresh, field-collected, air-dried and homogenized soil, equivalent to approximately 1kg oven-dry weight, is used in each microcosm. A series of four doses of the pesticide is applied to bulk batches of sieved soil with a fine sprayer, thoroughly mixed and homogenized. Six replicate microcosms are filled with treated soil for each dose of pesticide and controls, for each sampling date. All microcosms are kept at a temperature of 16-18°C in a continuous light chamber. Ten wheat seedlings are sown in the top 0.5 cm of the microcosm soil and, after 1-2 weeks, are thinned to 4 per microcosm.

In addition to the indigenous soil microorganisms, microarthropods, and nematodes present in the soil, 3 small earthworms of the species *Aporrectodea tuberculata* (Eisen.) (total weight of 1.5 g), or a similar species, common in most agricultural soils, are added to each microcosm.

At the bottom of each microcosm cylinder is placed a layer of mixed bed, ion exchange resins, separated from the bottom of the soil core by a thin layer of glass wool. This allows free passage of the soil leachate from the microcosm, and acts as a partial barrier to prevent root growth out of the core bottom, while collecting nutrient ions leaching from the microcosm.

Water is added to each microcosm two or three times a week to maintain a soil moisture content of 40-60% of field capacity (soil dry weight basis). Excess water is added once a week to leach through the soil core, as would occur under normal field conditions, and the leachate from each microcosm collected into a dish for nutrient and pesticide analysis.

Soil samples for microbial biomass, litter decomposition, enzyme activity, bait lamina tests, nutrient leaching and pesticide degradation, measurements are taken 0, 7, 14, 28 and 56 days after treatment. At the end of the experiment (56 days), microbial biomass, numbers of microarthropods, nematodes, and earthworms are assessed.

Terrestrial Model Ecosystem (TME)

The terrestrial model ecosystem consists of a 40 cm deep x 17.5 cm diameter soil core encased by a HDPE tube, which rests on an HDPE funnel with a thin layer of gauze between the funnel and the bottom of the soil core. Silicone tubing connects the funnel to an Erlenmeyer flask, which acts as a collection vessel. To obtain the cores, a specially designed steel extraction tube into which the empty HDPE tube is inserted and a hydraulic excavator or ram is used. Once the soil is cut by the leading edge of the extraction tube, a soil core is forced up into the HDPE tube using the hydraulic excavator. A ram is used to grip the extraction tube and to pull it together with the encased soil core very slowly from the ground. A HDPE cap is then fitted to the bottom of the soil core for transport to the laboratory.

In the laboratory, the terrestrial model ecosystems are placed into movable carts, which ensure that the soil temperature and the temperature for the leachate is significantly lower than the temperature above ground. A test is carried out to determine which sample model ecosystems to actually use in the study. For this test, a volume of artificial rainwater (Velthorst, 1993) which is approximately twice the average volume of water necessary to produce a breakthrough of leachate is applied to each model ecosystem using specially designed rain heads. After 48 hours the total volume of water collected is measured for each model ecosystem. Those model ecosystems that produce small or large volumes of leachate are discarded. The pesticide is applied to the soil surface as a liquid, or dry formulations and mixed with the upper soil layers to mimic agricultural application techniques.

The temperature inside the movable carts for the soil and the leachate is 12-15°C while the temperature in the growth chamber outside the movable carts is 20-22°C during the day (16 h, 12.000 - 16.000 lux) and 16-18°C during the night (8 h). The assessment of the weekly amount of artificial rainwater to be added to each soil core relates to the yearly precipitation in the site from which the soil cores were taken. The watering frequency should be at least once a week.

TEST DESIGN & MEASUREMENT OF ENDPOINTS

The pesticide doses applied to both ISM and TME are the recommended dose (T1), T1 x 6, T1 x 36, and T1 x 216. The various endpoints are measured 0, 7, 14, 28 and 56 days after application in ISM and minus 7 days, day 0, and 1, 4, 8 and 16 weeks after application in the TME. The number of replicates (usually 6) allows assessment not only of NOEC but also of EC_x values as discussed by the OECD (OECD, 1998). The high dosages for the test substance have been chosen to ensure that at this stage of the study clear dose-response relationships can be determined for several measurement end-points. It is not the intention of this preliminary test to mimic the effect and fate of carbendazim, applied at recommended application rates, but to assess the experimental appropriateness of the ISM and TME for prediction.

Table 1. Measurements in integrated soil microcosms (ISMs) and terrestrial model ecostystems (TMEs).

Measurement	Level II (SM)	Level III (TME)
Ecosystem Structure		
Microbial activity & diversity	Microbial biomass	Microbial biomass & diversity
Nematode communities	Numbers	Numbers in trophic groups
Earthworm Populations	Numbers & Biomass	Numbers, Biomass & Diversity
Microarthropod populations & diversity	Numbers & Biomass	Numbers, Biomass & Diversity
Enchytraeid populations	Numbers & Biomass	Numbers, Biomass & Diversity
Plant populations	Dry Weight Biomass	Dry Weight Biomass & Diversity
Ecosystem Processes		
Mineralization	Microbial Respiration	Microbial Respiration & Mineralization
Soil chemistry	C, N, P, pH	C, N, P, pH
Plant Nutrient Uptake	C, N	P,C, N
Organic Matter Decomposition	Loss from Litter Bags	Loss from Litter Bags
Biological Activity (Bait Lamina Test)	Loss of Organic Matter	Rate of Loss of Organic Matter
Fate of Pesticide		
Degradation pathways	Fate in Soil	Fate in Soil
Leaching	Amount Leached	Amount Leached
Uptake into earthworms	Amount in Earthworms	Amount in Earthworms
Uptake into plants	Amount in Test Plants	Amount in Indigenous Plants
Volatilization	Optional Test	Optional Test

Microbial Biomass: Microbial biomass is either measured as the C or N released from soil after chloroform fumigation (Brookes *et al.*, 1985) or by substrate induced respiration method (SIR) (Anderson & Domsch, 1978).
Microbial Enzyme Activity: Dehydrogenase enzyme activity is measured using calorimetric techniques (Frankenberger & Dick, 1983). Carboxymethylcellulase enzyme activity is measured as described by Eder (1993).

Nematodes and Enchytraeids: Nematodes and enchytraeids are extracted (wet extraction) and classified according to Edwards (1991) and Dunger & Fiedler (1997), respectively.
Microarthropods: Numbers, biomass and diversity are determined by using Tullgren funnels (dry extraction) according to Edwards (1991) and Van Straalen & Rijninks (1982).
Earthworms: Numbers and biomass are determined by hand-sorting (Edwards, 1991; Dunger & Fiedler, 1997). C, N and pesticides in tissues are analyzed.
Plants: The numbers of plants are counted at the end of the experiment, oven-dried at 65°C and weighed. In the TME either indigenous plants grow or cereals or legumes are sown.
Organic Matter Decomposition: In ISM organic material (chopped wheat straw), in a small cylinder of fiberglass screen (1.6 x 1.8 mm mesh), is inserted into the soil surface and periodic measurement of the rates of decomposition made by oven-drying and weighing. In TME either the litter-bags or filter paper is used (Dunger & Fiedler, 1997; Kula & Römbke, 1998).
Biological Activity: One bait lamina strip and four bait lamina strips are inserted into each ISM and TME, respectively, to assess changes in biological activity (Kratz, 1998).
Soil Chemistry and Nutrient Uptake by Plants: Soils and leaves are extracted with a salt solution (e.g., 0.5N K_2SO_4) for determination of the concentration of mineralizable nitrogen (Keeney & Brenner, 1966).
Fate of Pesticide: Samples are extracted with a solvent, cleaned up and analyzed, for the pesticide and degradation products, on a gas-liquid chromatograph or high performance liquid chromatograph.

CONCLUSIONS

The working program for the following two years is to use carbendazim as a model test substance in the ISM and TME, including a field validation study. The aim is to establish both microcosms as tools to assess ecosystem level parameters for the notification of chemicals and the registration of pesticides. We intend to identify trigger values from established effect and fate laboratory tests for use of microcosms. We will provide guidelines on using results from microcosms for environmental risk assessment procedures.

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Implications of a first-step environmental exposure assessment for the atmospheric deposition of pesticides in the UK

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ABSTRACT

The results of a desk-study carried out to review existing information on the aerial transport and deposition of pesticides in the UK were used to calculate worst-case aerial deposition loadings for three benchmark compounds with different patterns of detection. The loadings were used to carry out simple first-step calculations of predicted environmental concentrations in soil, surface water and groundwater. The results show that, in agricultural ecosystems, impacts of pesticides via aerial deposition are negligible compared to that from their direct agricultural application. For most compounds, aerial deposition in remote, natural or semi-natural ecosystems also results in negligible environmental exposure, although for some highly volatile, persistent compounds there appears to be the potential for more prolonged exposure, albeit at low concentrations. Future research should focus on developing methods to identify such compounds at an early stage of their development.

INTRODUCTION

Although first investigations on the presence of organochlorines in both air and rainfall were carried out in the 1960s by British scientists, few reliable UK data are available to clarify potential issues of regulatory concern relating to the long distance aerial transport and subsequent deposition of pesticides in the UK. Based on the results of a desk-study carried out to review existing information on the aerial transport and deposition of pesticides in the UK, this paper presents results of a first-step environmental exposure assessment for three benchmark compounds. Two, isoproturon and atrazine, showed a significant presence in the atmosphere only during the spraying season, whereas the other, lindane (γ-HCH), is the most frequently detected pesticide in rainfall and has been detected throughout the year in many instances.

MATERIALS AND METHODS

Evidence for Aerial Deposition in the UK

A total of ten papers (Wheatley & Hardman, 1965; Abbott *et al.*, 1965; 1966; Tarrant & Tatton, 1968; Wells & Johnstone, 1978; Clark & Gomme, 1991; Fisher *et al.*, 1991; Gomme *et al.*, 1991; Harris *et al.*, 1992) and six unpublished reports (Turnbull, 1989; Cranwell, 1992; Playford & Pomeroy, 1996; Preston & Merrett, 1993; Turnbull, 1995; Eastwood, 1995) that investigated the presence of pesticides in air, rainfall or snow in the UK were identified and reviewed (Dubus & Hollis, 1998a). For some pesticides, transient peak rainwater concentrations above the 0.1 µg/l EU directive limit for drinking water were detected, but these were short-lived and appeared to be rapidly diluted and dissipated. The highest concentrations detected in rainwater were for isoproturon and atrazine and were in the order of 1 µg/l. Organochlorines were always the most frequently detected pesticides in air and rainwater and were usually present at concentrations of a few to a few hundred ng/l throughout the year. For other compounds with lower vapour pressures, there is usually a correlation between their detection in rainwater and their agricultural spraying season. Some peaks which were detected outside the agricultural spraying season (Harris *et al.*, 1992) were attributed to non-agricultural usage of pesticides. Comparison of the UK data with published information from the rest of Europe (Dubus & Hollis, 1998b) reveals that measured pesticide concentrations in both rainwater and in air are broadly similar for both areas.

Estimation of worst-case environmental loadings from aerial deposition

Based on the limited evidence for aerial deposition of pesticides in the UK, a set of worst-case environmental loadings from rainfall were estimated for the aerial transport route for three 'benchmark' compounds, isoproturon, atrazine and lindane.

For pesticides for which detection is mainly limited to a few months in the year (isoproturon and atrazine), it was assumed that rainwater during the two most common months of application always contained pesticides at a concentration equivalent to the maximum concentrations detected in the UK studies. For pesticides that were found throughout the year (lindane), a mean annual concentration in rainfall was calculated, again based on measured data from UK studies. Figure 1 provides a graphical representation of the assumptions made.

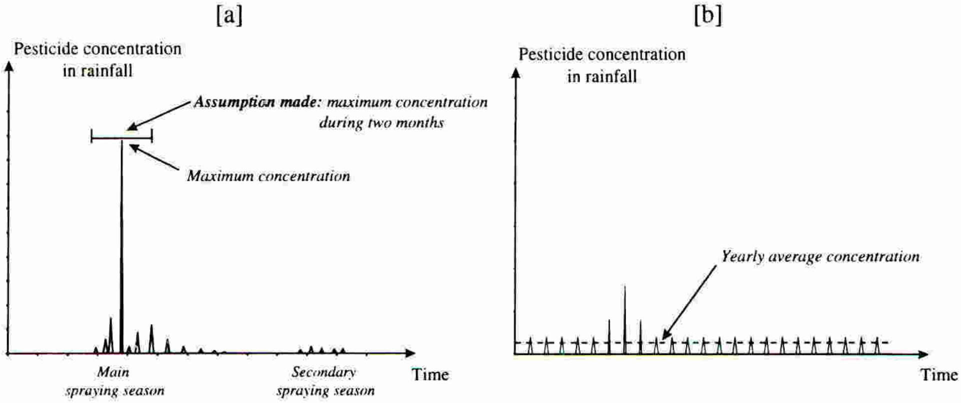

Figure 1. Type of deposition patterns considered (seasonal [a] and yearly [b] deposition).

The selected rainfall concentrations (see Table 1) were then used to calculate loadings resulting from a single rainfall event, the two months of rainfall during the main spraying season (for isoproturon and atrazine) and the total rainfall for one year (for lindane). Rainfall data for these calculations was selected from the 30-year weather dataset for Rosewarne held within the SEISMIC system (Hollis *et al.*, 1993). Rosewarne is located in Cornwall (grid reference 1463 0412) and has a long-term average annual rainfall of 1112 mm. It represents one of the climatically wettest agricultural areas of England. Using this dataset, the 75th-percentile wettest two months related to the main spraying season for isoproturon (October & November) and atrazine (April & May) and the 75th-percentile wettest year (for lindane calculations) were selected. Finally, the number of rainfall events during both the two-month period and the year were calculated as the 75th-percentile wet days. Details of the selected weather data and rainfall concentrations are given in Table 1.

Table 1. Summary of the main parameters used in the exposure assessment calculations.

Parameters	Units	Isoproturon	Atrazine	Lindane
Weather data				
Deposition period considered	-	Oct. / Nov.	Apr. / May	Year
75th percentile rainfall	mm	234.3	149.2	1184.1
75th percentile wet days	days	44.8	34.8	229.3
Number of rain events considered	-	45	35	229
Chemical data				
Koc	l/kg	35.3	39.8	207.9
DT50 soil	days	29	44	500
Deposition data				
Maximum concentration in rainfall [1]	ng/l	1550	69	-
Annual mean conc. in rainfall [1]	ng/l	-	-	55

[1] From Turnbull, 1995

Estimation of predicted environmental concentrations (PEC)

PECs in soil, surface water and groundwater were calculated after the first rainfall event containing pesticide, after the two months of rainfall related to the main application period (isoproturon and atrazine only) and one year after the first rainfall event. Calculations after two months were based on the following equation, proposed by the Soil Modelling group of FOCUS (EU, 1997) for calculating PEC following multiple applications:

$$PEC = PEC_{\text{after 1 application (rainfall event)}} \times (1 - e^{-nki}) / (1 - e^{-ki})$$

where k = degradation rate constant (ln2 / DT50)
n = number of applications (or rainfall events, for aerial deposition)
i = days between applications (or rainfall events, for aerial deposition)

PEC's for lindane, after one year were calculated using the same equation, whereas for isoproturon and atrazine, PEC's one year after the first rainfall were calculated, assuming no additional aerial inputs after the two month 'application period'.

Soil: PEC in soil was calculated using the simple first-step calculation guidelines provided by the Soil Modelling group of FOCUS (EU, 1997). Assumptions included first-order kinetics for degradation, no vegetation interception and a mixing depth of 5 cm. Average DT50's were taken from the PETE database (Nicholls, 1994) and the soil type was a sand of the Newport series with 1.1% topsoil organic carbon and an average bulk density of 1.5

g/cm^3 under arable cultivation. This data was taken from the databases held in SEISMIC (Hollis *et al.*, 1993).

Surface water: PEC calculations for surface water were made on the assumption of a direct overspray to a static ditch of 1 m width and 30 cm depth. Because of the lack of available information related to degradation in water or water/sediment systems, degradation in the ditch was considered to be the same as that in soil. Calculated PECs for surface water are therefore gross overestimates.

Groundwater: Rather than performing advanced simulation of pesticide transfer to groundwater using leaching models, a simple first-step assessment was conducted. The concentrations of pesticide in soil water in the first 5 cm were computed using the Freundlich isotherm applied to the mass of pesticide present in the soil as calculated from the estimated PEC in soil. Koc values were taken from the PETE database (Nicholls, 1994). This is clearly an unrealistic worst-case assessment for groundwater, but provides a first-step estimation for evaluation purposes.

RESULTS

Comparison of aerial deposition loadings with those from normal agricultural applications

Table 2 shows that, even using the worst-case assumptions for aerial deposition of the compounds, the calculated maximum environmental loading from aerial deposition is several orders of magnitude lower than the loading immediately following application at the manufacturers' maximum registered rate.

Table 2. Environmental loadings (g/ha) of three benchmark compounds from aerial deposition and from direct agricultural application.

Compound	Isoproturon	Atrazine	Lindane
Maximum registered agricultural loading	2500	1300	1120
Aerial deposition for a single rainfall event	0.081	0.003	0.003
Cumulated aerial deposition after 2 months (during the main spraying season)	3.632	0.103	N/A
Cumulated aerial deposition after 1 year	N/A	N/A	0.651

PECs for soil, surface water and groundwater

Table 3 shows the calculated PECs resulting from the aerial deposition of each benchmark compound. A comparison is made with PECs calculated using the same equations, but based on loadings from a direct agricultural application. For all three environmental compartments, the predicted maximum concentrations arising from aerial deposition of the benchmark compounds suggest an insignificant level of environmental exposure. Even for groundwater where the unrealistic worst-case nature of the calculations gives the highest values, PEC's are several orders of magnitude smaller than those resulting from a direct agricultural application.

Table 3. Predicted environmental concentrations (overestimates) of three benchmark compounds resulting from aerial deposition and direct agricultural application.

PEC	Isoproturon			Atrazine			Lindane		
	Soil mg/kg	S/Water µg/l	G/Water µg/l	Soil mg/kg	S/Water µg/l	G/Water µg/l	Soil mg/kg	S/Water µg/l	G/Water µg/l
Ag 1	3.3E+00	8.3E+02	6.3E+03	1.7E+00	4.3E+02	3.0E+03	1.5E+00	3.7E+02	6.2E+02
Ad 1	1.1E-04	1.1E-02	2.0E-01	3.9E-06	3.9E-03	6.8E-06	3.8E-06	3.8E-03	1.6E-03
Ad 2m	2.6E-03	6.5E-02	3.8E+00	8.9E-05	2.2E-03	1.2E-01	N/A	N/A	N/A
Ad 1y	2.0E-06	4.5E-04	2.7E-03	7.9E-07	1.9E-04	1.0E-03	7.0E-04	1.1E-02	2.7E-01
Ag 1y	6.1E-04	1.4E-02	8.0E-01	6.0E-03	1.4E-01	7.6E+00	9.1E-01	2.5E+02	5.3E+02

Ag 1: directly after agricultural application; *Ad 1*: directly after the first rainfall deposition event; *Ad 2m*: after two months of aerial deposition; *Ad 1y*: after one year of aerial deposition; *Ag 1y*: one year after a direct agricultural application.

CONCLUSION

The results of this first-step worst-case assessment, based on unrealistically extreme assumptions, show that the environmental impact of pesticides deposited from the atmosphere onto agricultural ecosystems is negligible compared to direct agricultural application. Even in natural and semi-natural ecosystems, most pesticides deposited via the atmosphere will have negligible environmental impact because of the very small concentrations involved and their rapid dissipation in soil and water. However, in the opinion of the authors, a concern remains with respect to highly volatile, persistent compounds, especially those which have the potential to bioaccumulate (e.g. lindane). In such cases, long-distance transport to remote areas could lead to their significant accumulation within natural or semi-natural ecosystems. Future research should focus on developing relationships between national pesticide usage, physico-chemical properties and atmospheric persistence, so that compounds with the potential for long-distance aerial transport and accumulation can be identified at an early stage of development.

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The poisoning of animals from the negligent use of pesticides

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ABSTRACT

The Wildlife Incident Unit (WIU) of the Central Science Laboratory (CSL) analyses a wide range of samples for pesticide residues and interprets the results in support of the Wildlife Incident Investigation Scheme. The Scheme reports on the suspected poisoning of wildlife, companion animals, and bees by agricultural chemicals. Over three years, from 1994 to 1996, there were 408 suspected pesticide poisoning incidents in England. In 76 of these incidents the pesticides were used negligently. The source of the pesticide was not certain in another 95 incidents and some of these may also have been due to negligent use. Vertebrate control products and molluscicides were principally associated with negligent use incidents and dogs were most frequently affected. Spillages and exposed baits account for the majority of these incidents. Spray applications during flowering and poorly conducted feral bee treatments were the main causes of bee incidents.

INTRODUCTION

The Wildlife Incident Investigation Scheme (WIIS) reports on the suspected poisoning of wildlife, companion animals and bees by agricultural chemicals. For more information on the operation of the Scheme and annual results refer to Fletcher *et al*. (1997) and previous reports in this series. There have also been some reviews of the results of the Scheme (Hardy *et al.*, 1986; Greig-Smith *et al.*, 1994; Barnett *et al.*, 1997).

In recent years, the rigorous approval process for pesticides has meant that problems following the proper use of pesticides are rare (Fletcher & Grave, 1992). The majority of poisoning incidents continue to be due to the intentional illegal use of pesticides. In most of the remaining incidents there is evidence that the label instructions have not been followed or the source of the pesticide is uncertain. Often the negligent use of a pesticide is accidental, and in some instances it may reveal a lack of clarity in label instructions. This paper will focus on the incidents resulting from negligent use for the years 1994 to 1996.

METHODS

Field information on the circumstances of an incident is obtained by the Farming and Rural Conservation Agency (FRCA). This field enquiry is carried out as soon as possible after the incident is reported to them. The Veterinary Investigation Centres of the Veterinary Laboratories Agency (VLA) undertake most of the gross post-mortems for the Scheme.

The analytical methods used by the WIU are reported in Brown *et al.* (1996). They are validated for the detection of the parent compound in animal tissues, but baits and formulation samples are also analysed. Toxicity data, post-mortem findings and experience of previous incidents are used to interpret the significance of residues.

Every poisoning incident is assigned to a category of pesticide use and these are: **Approved use** of a product, according to the specified conditions for use; **Misuse** (or negligent use) of a product, by careless, accidental or wilful failure to adhere to the correct practice; **Abuse** of a pesticide, in the form of deliberate, illegal attempts to poison animals; **Unspecified use**, where the cause could not be assigned to one of the above categories.

RESULTS

In England and Wales for the three years from 1994 to 1996 there were a total of 447 suspected pesticide poisoning incidents. However, there were no incidents of pesticide misuse in Wales, so only incidents in England will be reviewed. Over a thousand incidents have been investigated during this time (Table 1) and pesticide poisoning confirmed in 33% of these incidents. Just over a half of these poisoning incidents were from the intentional illegal abuse of pesticides. Misuse of pesticides occurred in 19% of incidents and unspecified use in 23% of incidents. It is likely that in some unspecified use incidents, where the source of the pesticide has not been established, misuse has occurred. Only 7% of pesticide related incidents were suspected to have resulted from approved use.

Table 1. Incidents investigated and pesticide poisonings in England 1994-1996.

Year	Number of incidents investigated	Number of pesticide poisonings	abuse	**misuse**	approved	unspecified
1996	354	129	77	**20**	10	22
1995	433	143	70	**31**	6	36
1994	454	136	63	**25**	11	37
TOTAL	**1241**	**408**	**210**	**76**	**27**	**95**

The pesticides and the species affected in misuse incidents

There have been 23 different pesticides identified in the 76 misuse incidents (Table 2). Just over a half of the incidents occur through the use of vertebrate control products. Nearly a quarter of these incidents include the compounds alphachloralose, calciferol, strychnine, aluminium phosphide and sodium cyanide and the rest involve anticoagulant rodenticides. Bromadiolone, warfarin and difenacoum account for the majority of the rodenticide incidents and the species most commonly affected are dogs, cats and badgers. Molluscicides were involved in 18% of misuse incidents and dogs, particularly labradors, were the species most commonly affected. There was one incident where a badger was exposed to metaldehyde. Most of the incidents involved metaldehyde, except two incidents with methiocarb.

Table 2. Pesticides and the samples / species involved in misuse incidents 1994-1996.

Pesticides	Number of incidents	Samples / species involved in incidents
Anticoagulant Rodenticides		
mixture of rodenticides*	9	grain / cat, chicken, dog, feral pigeon.
bromadiolone	9	grain / chicken, feral pigeon.
warfarin	5	grain / badger, cat, dog, grey squirrel.
coumatetralyl	3	grain / cat, dog, pig.
difenacoum	2	grain / dog.
brodifacoum	1	grain / dog.
chlorophacinone	1	grain / dog.
Molluscicides		
metaldehyde	12	pellets / badger, cat, dog.
methiocarb	1	pellets / dog.
mixture of molluscicides	1	pellets / dog.
Carbamates		
bendiocarb	10	honeybee: affected 36 colonies.
carbaryl	1	honeybee: affected 3 colonies.
Organophosphates		
dimethoate	4	honeybee: affected 66 colonies.
chlorpyrifos	2	honeybee: affected 13 colonies.
fenitrothion	1	honeybee: affected 8 colonies.
Others		
alphachloralose	5	pigeon carcase, powder sample / feral pigeon, kestrel, peregrine, starling.
chlordane	2	jackdaw, kestrel, starling.
mixture of compounds**	2	pellets / fox.
aluminium phosphide	1	badger, badger sett.
lambda cyhalothrin	1	bumble bee.
paraquat	1	horse.
sodium cyanide	1	badger sett.
strychnine	1	soil.
TOTAL bees	**19**	
other	**57**	

* Two incidents difenacoum/warfarin; and single incidents with: bromadiolone/difenacoum; bromadiolone/coumatetralyl; bromadiolone/warfarin; difenacoum/coumatetralyl; chlorophacinone/alphachloralose; chlorophacinone/bromadiolone; warfarin/calciferol.

** Single incidents with: carbofuran/metaldehyde and paraquat/diquat.

Carbamate and organophosphate insecticides account for most of the remaining incidents and these all involved honeybees. A spray application of lambda-cyhalothrin also affected 15 bumble bees. A field was oversprayed with paraquat and a horse suffered lung problems and tongue blisters. There was also one incident where three foxes died following an application of paraquat and diquat. A jackdaw, two starlings and a kestrel probably died from an application of chlordane on a golf course.

Misuse scenarios

Rodenticides and other vertebrate control products (38 incidents)

Baits which were accessible to non-target species caused most of the anticoagulant rodenticide incidents. Often no dead, or ill animals were found, but where baits are used in this way it increases the risk of exposure for non-target species. Sometimes bait was completely exposed, such as grain left on a river bank, grain in rat burrows, in hedgerows, or in large open trays in farm buildings. Plastic packets of rodenticide bait used unprotected can also place other animals at risk. The use of hay bales is often encountered as a means of protecting the bait. The gaps between the bales are usually too large, or when bales are removed the bait is left uncovered. Lengths of pipe or bait covered with a roof tile are often used rather than proper bait boxes. This improvised protection of bait is only acceptable if other precautions are followed. Enquiries made during these investigations have revealed that accurate and complete treatment records may not have been kept, bait may not have been removed following the completion of a treatment and rat carcases may not have been actively looked for, removed and burnt. Rodenticide treatments may also have been undertaken by people who have received no training in their correct use.

Brodifacoum is only registered for use indoors and bait should only be placed within a building or enclosed structure that has rats living or feeding predominantly within that building or structure. In one incident investigated, a dog was seriously ill as the bait had been used outside and had been placed in a hopper with a spillage of grain surrounding it.

Alphachloralose misuse occurs through bird narcotic treatments undertaken without a properly authorised licence. A peregrine died, probably from eating poisoned pigeons and starlings were also affected. A kestrel was found slumped over a pigeon carcase, it recovered after treatment. In one incident alphachloralose was found in an unlabelled container in the back of a gamekeepers vehicle. Strychnine was used on residential land for mole control, but this use is only permitted on agricultural land. Two gassing incidents were misuse, as the operatives failed to identify badger setts which were gassed during rabbit control treatments.

Molluscicides (15 incidents)

Metaldehyde and methiocarb are the molluscicides involved and metaldehyde is consistently identified in more incidents than any other pesticide detected by the Scheme. The most usual occurrence is spillages that are not cleared away. Spillages often occur on headlands where application equipment is turned, or they are the result of faulty applicators and careless filling of equipment. Poor storage of slug pellets provides an opportunity for animals to be exposed, particularly where open bags are left in barns or open pesticide stores. If pellets become damp from poor storage they are sometimes dumped or sprayed on a field and as the pellets clump together they again pose a risk to wildlife or pets. Mixtures of compounds were found in two incidents. One involved a dog that was poisoned from a spillage of metaldehyde and methiocarb and the other was metaldehyde and carbofuran stored together in an unlabelled jar.

Insecticide sprays (8 incidents)

All the misuse incidents occurred as crops in flower or crops with flowering weeds present were sprayed. Seven of these incidents involved honeybees and one incident bumble bees. The honeybee deaths occurred following insecticide spray applications to flowering: oilseed rape (three incidents); raspberries (two incidents) beans (one incident); and an orchard (one incident). Dead bumble bees were found after the synthetic pyrethroid, lambda-cyhalothrin, had been applied to a flowering broad bean crop.

Feral bee treatments (11 incidents)

Bendiocarb is usually involved, but there was one incident with carbaryl. The main reasons these incidents occur and breach the label conditions is because treated comb is not removed and/or the treated area is not properly sealed to prevent access by robbing honeybees. The scenarios often encountered are treatments undertaken in roof voids, chimneys and old tree stumps, which are all difficult to seal off adequately. An amateur product containing bendiocarb, only registered for use against crawling insects, was used to control feral bees in one incident.

Other uses (4 incidents)

In two separate incidents, the death of a jackdaw, two starlings and a kestrel were attributed to exposure to chlordane. Soil samples also contained chlordane. This pesticide was banned in 1992 and it seemed likely that chlordane had been applied after this date. The herbicide incidents resulted from overspray with paraquat into a field where a horse was kept and in the other incident the paraquat and diquat were mixed at four times the recommended strength.

DISCUSSION

Vertebrate control products are the most frequently misused pesticides and anticoagulant rodenticides account for the majority of these incidents. These products have a widespread usage, which may partly explain the number of incidents which occur. The risk of exposure and death of non-target animals is greatly increased by the misuse of these products. Dogs are often associated with these incidents, but there is evidence of exposure and cause of death in a number of wild mammal and bird species. For example, the Scheme has found lethal residues of anticoagulants in goshawk, red kite, kestrel, polecat, badger and fox. The bird species are exposed via eating poisoned rats or non-target species, whereas the mammals may also be eating the bait directly. From WIIS data, mammal incidents with anticoagulant rodenticides are more common than bird incidents. More than one rodenticide was found in nearly a third of these incidents, with difenacoum and warfarin the most frequent combination. However, bromadiolone is consistently found in more incidents than the other rodenticides. Greater publicity of the potential dangers of negligent rodenticide treatments is required.

Metaldehyde is widely available in amateur and professional use pellet products. Due to the high number of reported misuse incidents involving companion animals action has been taken. This action includes label changes and increased product stewardship highlighting the importance of responsible use and storage of these products.

Six bee incidents were classified as pesticide misuse in 1994, compared to twelve in 1995 and one in 1996. Incidents with organophosphates are declining and this may partly be due to an increased use of synthetic pyrethroids which are approved for use on specified flowering crops. In organophosphate spray incidents, generally all the colonies in an apiary are affected, whereas in feral bee treatments it is typical for only one or two colonies in an apiary to be affected. Education campaigns on feral bee treatments appear to have been successful, as for the last few years there have only been one or two honeybee mortality incidents from this use. However, varroa mites may have reduced feral bee numbers and the need for these treatments.

Investigations of misuse incidents have led to many successful prosecutions. The WIIS continues to provide reassuring evidence of a successful registration process, due to the low numbers of incidents reported from the approved use of pesticides and provides valuable information to the regulatory authorities in the UK.

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Spray drift into field margins: The effect of width of buffer strip and plant species on the interception of spray drift

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ABSTRACT

The spray-tracer dye, fluorescein, was used to measure the amount of spray drift intercepted by 16 species of commonly occurring arable field margin plant protected by 6m and 2m wide buffer strips compared with plants adjacent to a fully sprayed strip. Whereas the amount of spray drift was significantly reduced on plants protected by the 2m and 6m wide buffer strips, plants did not receive significantly less drift in the 6m than in the 2m wide strips. Spray drift interception differed between plant species and hairy leaved plants received significantly less spray drift that non-hairy leaved plants. Plant leaf area was shown not to influence the amount of spray drift intercepted.

INTRODUCTION

Pesticide use has been cited as one of the most significant factors in the decline in biota in arable land (de Snoo, 1994) and is also attributed to increasing the problem of some aggressive weed species and making their control ever more difficult (Marshall & Birnie, 1985). While it is known that the direct control of pest arthropods and weeds leads to species-poor communities (Chiverton & Sotherton, 1991), the effects of pesticides on non-target biota in field margins and buffer strips are less easy to determine. Buffer strips are an ideal method of increasing the distance from the sprayer to non-target areas and have been shown to significantly reduce the amount of pesticide reaching non-target biota (Breeze *et al.*, 1992). Questions still remain about appropriate widths, since recommendations vary from 2m to 20m (Marrs *et al.*, 1989; Marrs *et al.*, 1993), however, it is in the interests of agricultural production to keep widths to a minimum.

Because of the extent of their surface area, plants receive more pesticide than any other organism, which may then be accessible to locally occurring fauna. The interception of pesticide drift, especially herbicide, by various non-crop plant species is important in terms of conservation of on-farm biodiversity, due to both the direct toxicity effect of herbicides and indirect effects: the availability of food plants and structural features may be diminished. Potential implications for disruption to taxa in the higher trophic levels may exist.

Research into herbicide spray drift has recommended buffer strips of at least 2m wide for protection against lethal damage to plants caused by most herbicides (Marrs *et al.*, 1989), but measurement of drift on individual plant species has received little attention. In this study, we attempt to determine the effectiveness of different widths of buffer strip in protecting various plant species from medium quality spray drift. The effects of plant species, leaf area and leaf texture on the interception of spray are also investigated.

MATERIALS AND METHODS

Sixteen species of plant, which commonly occur in arable field margins (grasses and forbs) were raised from seed in 10cm pots (Table 1). The plants were grown for 6 months prior to being sprayed with a tracer dye, and this permitted healthy development of each plant.

Table 1. Grass and forb species and corresponding leaf texture.

Species	Texture	Species	Texture
Elymus repens (*Er*)	non-hairy	*Cerastium holosteoides* (*Ch*)	hairy
Festuca rubra (*Fr*)	non-hairy	*Geranium robertianum* (*Gr*)	hairy
Lolium perenne (*Lp*)	non-hairy	*Rumex obtusifolius* (*Ro*)	non-hairy
Dactylis glomerata (*Dg*)	non-hairy	*Lamium album* (*La*)	hairy
Arrhenatherum elatius (*Ae*)	non-hairy	*Tripleurospermum maritimum* (*Tm*)	non-hairy
Agrostis stolonifera (*As*)	non-hairy	*Cirsium vulgare* (*Cv*)	hairy
Silene alba (*Sa*)	hairy	*Cirsium arvense* (*Ca*)	non-hairy
Stellaria media (*Sm*)	hairy	*Centaurea nigra* (*Cn*)	hairy

Each plant species was randomly allocated a position adjacent to a fully sprayed strip, 2m and 6m wide buffer strips, and placed 0.5m apart so that there was no between-plant contact. These positions were maintained for each species in the 5 replicates of the experiment, using new plants for each replicate. Figure 1 illustrates the experimental layout in the field, where species are represented by their name abbreviation (refer to Table 1).

The spray tracer dye, sodium fluorescein (1g in 10 litres water + 0.1% v/v Agral), was applied at a standard volume of 265 l/ha from a tractor-mounted sprayer fitted with Hardi 4110-20 nozzles at spray pressure 3 bar and a forward speed of 2m/s. Four double passes of the strips were made to reduce the effect of inherent variability of spray within drift clouds. The 12m boom was positioned 0.5m above plant height and spraying took place at wind speeds between 1.2 and 2.2 m/s. Fluorescein was washed off 2 leader leaves in standard non-ionic buffer solution (50ml water + 0.1% v/v Agral) and tracer concentrations were analysed using a luminescence spectrometer (Perkin Elmer LS30). Leaf areas were measured to allow the calculation of drift as µl/mm.

Field		
Plant position sprayed	*Ae Ca Er Sa La Ch Ro Fr Cn Dg Tm Cv Lp Gr Sm As*	Fully
Buffer Areas	*Ae Ca Er Sa La Ch Ro Fr Cn Dg Tm Cv Lp Gr Sm As*	2m
	Ae Ca Er Sa La Ch Ro Fr Cn Dg Tm Cv Lp Gr Sm As	6m

Figure 1. Configuration of the 16 plant species in fully sprayed, 2m and 6m wide buffer strips. Refer to table 1 for key to abbreviations.

Data were log (x +1) transformed and two-way ANOVA, with plant species and width of buffer strip as factors, were used to test for significant differences between treatments and interactions between plant species and width of buffer strip. Planned comparison tests (LSDs) at 95% probability were used to test for differences between means implicit in the experimental design (Sokal & Rohlf, 1995).

RESULTS

Plants received significantly different amounts of spray drift per mm^2 at each of the three buffer strips ($F_{(2, 192)} = 63.77$, $P<0.0001$). Plants adjacent to the fully sprayed strip received significantly more spray drift than those at the 2m and 6m wide strips, while plants protected by the 2m and 6m buffer strips did not receive different amounts of spray drift from each other (Table 2).

Table 2. Mean deposit of drift (µl/mm) on plants in buffer strips and LSD value.

Buffer Strip			LSD
Fully Sprayed	2m	6m	
0.0312	0.0045	0.0012	0.0138

The amount of spray drift intercepted per mm^2 by plants differed between species ($F_{(15,192)} = 6.83$, $P<0.0001$): *F. rubra*, *A. elatius*, *G. robertianum* and *R. obtusifolius* intercepted significantly greater amounts of drift than the other species (Table 3).

Table 3. Mean deposit of drift (μl/mm) on different plant species and LSD value. (Refer to Table 1 for key to abbreviations).

Plant Species & Mean Deposit								LSD
Ae	Ca	Er	Sa	La	Ch	Ro	Fr	
0.0426	0.0051	0.0039	0.0028	0.0038	0.0025	0.0324	0.0312	0.0060
Cn	Dg	Tm	Cv	Lp	Gr	Sm	As	
0.0088	0.0148	0.0062	0.0107	0.0033	0.0177	0.0070	0.0034	0.0060

There was a significant interaction between plant species and width of buffer strip indicating that the amount of spray intercepted per mm^2 varied between species within each strip ($F_{(30, 192)}$ = 4.01, P<0.0001). Nine plant species were found to receive significantly different amounts of spray drift to other species at both the fully sprayed and the 2m wide strips, while only 7 plant species were found to receive significantly different amounts of drift per mm^2 adjacent to the 6m wide buffer strip (refer to Table 4).

Table 4. Mean deposit of drift (μl/mm) on plant species in different buffer strips, refer to Table 1 for key to abbreviations. Mean deposit values are x 10^{-3}. Percentage deposits of 0m values are given in parentheses. LSD = 0.0229

Species	Ae	Ca	Er	Sa	La	Ch	Ro	Fr
0m	101	13.8	9.2	6.7	8.5	7.1	88.9	76.8
2m	22.6 (22)	1.1 (8)	1.9 (21)	1.4 (21)	2.1 (25)	0.3 (4)	5.5 (6)	15.4 (20)
6m	4.1 (4)	0.4 (3)	0.5 (5)	0.5 (7)	0.7 (8)	0.1 (1)	2.7 (3)	1.4 (2)
Species	**Cn**	**Dg**	**Tm**	**Cv**	**Lp**	**Gr**	**Sm**	**As**
0m	22.0	24.0	15.6	22.9	7.9	49.8	18.7	10.4
2m	2.8 (13)	8.4 (35)	2.0 (13)	2.3 (10)	1.6 (20)	1.9 (4)	1.6 (9)	0.9 (9)
6m	1.6 (7)	2.0 (8)	1.1 (7)	1.0 (4)	0.4 (5)	1.3 (3)	0.6 (3)	0.3 (3)

When drift deposition per mm^2 was compared for individual species across each of the three strips, it was shown that *D. glomerata* received similar amounts at the fully sprayed and 2m wide buffer strips, but significantly more drift adjacent to the fully sprayed strip than at the 6m wide buffer. *C. vulgare* received similar amounts of drift per mm^2 at all three strips. All other species received significantly greater amounts of drift per mm^2 adjacent to the fully sprayed strip than at both the 2m and 6m wide buffer strips. For all species, there was no significant difference between the amount of deposition per mm^2 at the 2m wide strip and the 6m wide strip.

There was no relationship between leaf area and the amount of spray drift (r^2 = 0.007, P<0.0954), indicating that larger leaves do not intercept more spray per unit area than smaller leaves. However, texture of leaves did influence the amount of spray drift intercepted, with non-hairy leaves receiving more spray than hairy leaves ($F_{(1, 238)}$ = 5.38, P<0.0212).

DISCUSSION

The results from this experiment show that even relatively narrow buffer strips can significantly reduce the amount of spray drift reaching non-target plants. When compared with the amounts of spray in the fully sprayed strip, the 2m buffer strip reduced the amount of spray per mm^2 of leaf by an average of 85%, while the 6m buffer strip reduced this further to 95%. Surrounding vegetation is thought to offer protection from pesticide drift (Marrs *et al.*, 1991) and a higher and more dense vegetation cover has been shown to intercept more drift (Marrs *et al.*, 1993). Since this study was carried out in rough grassland, where the experimental plants were equal to or taller than the surrounding vegetation the plants probably intercepted the maximum drift possible.

Although effective for most plant species, the 2m wide buffer strip did not provide significant protection from spray drift for *Dactylis glomerata* and *Cirsium vulgare*. Furthermore, *C. vulgare* was not significantly protected from spray drift by even the 6m wide strip, possibly due to a combination of plant height and its leaves being angled perpendicular to the direction of the drift. Despite 6m wide buffer strips being suggested for avoiding lethal effects of spray drift (Marrs *et al.*, 1989; Marrs *et al.*, 1991), this experiment illustrates that plants still receive measurable amounts of spray even in the 6m buffer. The biological impact of drift droplets depends on the sensitivity of field margin plant species (Davis *et al.*, 1994) and plants protected by 6m wide buffer strips may still suffer lethal effects when high doses of herbicide are applied. Marrs *et al.*, (1993) found that seedlings of some species were sensitive to glyphosate spray drift up to 20m down-wind of the sprayer. Thus, where species establishment is important, buffer strips may need to be increased to greater than 6m wide.

Four species intercepted significantly greater amounts of spray drift than others (*F. rubra*, *A. elatius*, *G. robertianum* & *R. obtusifolius*). These plants provide general habitat structure, important for web-building spiders (Alderweireldt, 1994) and food plant material for some invertebrates such as Tingidae and Stenodemini (Insecta: Heteroptera) (Southwood & Leston, 1959). Interception of large amounts of agrochemical drift at fully sprayed field margins may have implications for these non-target invertebrates.

Leaf area was not important in determining the amount of spray intercepted by a plant, however texture was: plants with hairless leaves intercepted significantly more drift than those with hairy leaves. It may be that hairs on leaves act as a shield for the leaf surface and could limit the adsorption of agrochemical, thus, non-hairy leaved plants may be more sensitive to the effects of agrochemical drift. *D. glomerata* is a hairless, relatively tall grass and these factors combined may have contributed to this species being more sensitive to drift than other species at the 2m buffer strip. Although *C. arvense* is hairy-leaved, it is a tall plant and was likely to have been more exposed to spray drift even at the 6m wide buffer strip.

This study shows that plant species vary in their ability to intercept spray drift, due to their leaf texture, and other factors, such as plant height and orientation of leaves. It is therefore not easy to decide widths of buffer strips or field margins, however, we suggest a 2m wide strip, which appears to protect most plant species tested here from significant amounts of spray drift, could be easily implemented.

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Considerations with the use of multiple dose bioassays for assessing pesticide effects on non-target arthropods

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ABSTRACT

In principle, laboratory bioassays of pesticides against non-target species conducted at a range of doses or concentrations offer considerable benefits over ones at the recommended field application rate alone. This paper describes bioassays with dimethoate against predatory ground beetles (Coleoptera: Carabidae) and wolf spiders (Araneida: Lycosidae) undertaken to explore the feasibility of obtaining dose-response data for predatory arthropods, and the influence of biological variables on the precision of bioassay results. In general, the tests showed good repeatability, and results showed an adequate fit to the probit regression model. Individuals of *Pterostichus cupreus* L. captured in the field or reared in the laboratory responded identically to dimethoate. Although females of both *P. melanarius* Illiger and *P. madidus* Fab. proved slightly (*c.* 2-fold) more tolerant than males, this difference is unlikely to impair severely the accuracy or interpretion of results. All five species tested including three species of *Pterostichus*, the smaller carabid *Nebria brevicollis* Fab. and the lycosid *Trochosa ruricola* Degeer yielded similar LC_{50} estimates for dimethoate that were 2- to 4-fold less than the recommended field concentration. The implications of these findings for ecotoxicological risk assessment programmes are discussed.

INTRODUCTION

Despite widespread concern over possible effects of pesticides on non-target species, approaches for evaluating such risks and incorporating them into registration procedures are still hotly debated. It is generally accepted, however, that a risk assessment scheme should encompass a succession of tests of increasing scale and complexity. This has become obligatory in several countries for the registration of plant protection products (Europe, 1991).

The first stage of testing is usually designed to represent a 'worst case scenario' (Barrett *et al.*, 1994; Hassan *et al.*, 1994). This will invariably be laboratory based, with the emphasis on precision and reproducibility rather than realism. The current practice advocated by the 'Pesticides and Beneficial Organisms' Working Group of the International Organisation for Biological Control (IOBC) and other bodies is to test individuals at a single dose or concentration corresponding to the maximum envisaged field rate for a given pesticide (Hassan *et al.*, 1994). The observed percentage mortality is used to place chemicals into categories that may or may not trigger a move to the next stage in the testing heirarchy.

Single-dose tests have operational advantages, but can be criticised in at least two respects. Firstly, they provide no information on the distribution of tolerances with populations of non-target organisms, which could be used, for example, to anticipate the consequences of changing application dosages to contend with different crops or pest species. Secondly, mortality estimates at a single dose (especially if close to 0 or 100%) are subject to statistical constraints and/or unexpected sources of variation that limit meaningful comparisons between data for different chemicals or species (Denholm *et al.*, 1998). The use of multiple dose bioassays to characterise dose-response relationships could, in principle, overcome these limitations, but so far there have been very few studies (e.g. that of Cilgi *et al.*, 1996) on the utility of incorporating this approach into risk assessment schemes for non-target arthropods.

Using data for predatory ground beetles (Coleoptera: Carabidae) and wolf spiders (Araneida: Lycosidae), important generalist predators within arable ecosystems, we describe here work to investigate the feasibility of obtaining dose-response data for predatory arthropods, and the effects of biological variables including rearing history and sex on the precision of bioassay results. The practical implications of these findings are discussed in light of logistical constraints and the scientific goals of risk evaluation procedures.

MATERIALS AND METHODS

Arthropod species and culture conditions

Data presented below refer to four species of carabid beetle, *Pterostichus (=Poecilus) cupreus* L., *Pterostichus melanarius* Illiger, *P. madidus* Fab. and *Nebria brevicollis* Fab., and one species of lycosid spider, *Trochosa ruricola* Degeer. All were collected from the field in dry pitfall traps and kept in the laboratory for a minimum of one week before being used in bioassays, in order to avoid using individuals in a poor state of nutrition. In addition, laboratory-bred individuals of *P. cupreus* were obtained from Dr Udo Heimbach, BBA, Braunschweig, Germany.

Beetles were sorted by species and sex and kept in mesh-covered trays (approx. 35 x 50 x 15 cm) containing *c.* 4 cm of loosely compacted damp sand. Stones or broken clay plant pots were placed on the sand for shelter. Densities were kept below 40 individuals per tray to minimise cannibalism. Trays were stored at 21°C ± 1°C under a 16 h photoperiod. Beetles were fed on dried cat biscuits (Sainsburys' 'Paws Complete') that had previously been soaked in water. Male *T. ruricola* were kept individually, to prevent cannibalism, in small plastic containers (7.5 x 2 x 4.5 cm) lined with 1 cm of Plaster of Paris containing ground charcoal. The spiders were stored in a constant environment room at 3°C ± 1°C with low light and fed on frozen adults of *Drosophila* species. Four days prior to bioassays, spiders were moved gradually to 10°C ± 1°C, 18°C ± 1°C and finally 21°C ± 1°C, the temperature at which bioassays were conducted.

Insecticide

The insecticide used was the organophosphate dimethoate, applied as formulated product ('Atlas Dimethoate 40' EC) in distilled water using a hydraulic track sprayer at the equivalent of 200 litres/ha under a pressure of 3 bar at 35cm above the ground.

Bioassay Method

The bioassay method was based on that described for *P. cupreus* by Hassan (1985). For this work, however, the substrate was sand instead of soil, and the test organisms were sprayed as well as the substrate. Ten beetles or spiders placed in a plastic container (17 x 11.5 x 6.5 cm) containing dry sand were sprayed with each concentration of insecticide. Test organisms were then transferred individually into plastic cups containing moist sand (200 ml water per litre of sand) that had been sprayed with the same concentration of dimethoate. Bioassays were stored at 21°C ± 1°C, and mortality assessed one, three and seven days after spraying. During this period, spiders were fed on *Drosophila* adults and beetles on blowfly (*Calliphora* spp.) pupae. Controls were set up and maintained in an identical manner, but were left unsprayed.

For the purposes of the assessment, mortality is defined as both dead individuals and ones incapable of co-ordinated movement seven days after treatment. This is valid for dimethoate since, in our experience, symptoms of poisoning by this chemical are irreversible. Although these graphs refer to single bioassays, each was conducted a minimum of three times to assess repeatability and, when appropriate, data from replicate tests were pooled for probit analysis using the POLO programme (LeOra Software Inc. Berkeley, CA).

RESULTS AND DISCUSSION

In general, bioassays showed good repeatability and clear cut relationships between mortality and spray concentration. Although individual tests sometimes gave poor fits to the probit regression model due to erratic fluctuations in mortality and/or insufficient data points, pooled results for replicate bioassays yielded relatively steep lines (slope values > 2) with 95% confidence limits on fitted LC_{50} values spanning less than a two-fold range of concentrations (Table 1). Failure in some cases to calculate 95% limits was due to replicate tests spanning different concentration ranges, resulting in too few measurements of intermediate mortality that carry most weight in probit analysis (Finney, 1971). If probit analysis is the primary objective, choice of concentrations is critical (e.g. Robertson *et al.*, 1984), and this will require preliminary tests to optimise the number and range of those applied. However, accurate line-fitting is by no means essential for disclosing dose-response relationships or for comparing results for different chemicals or taxa, since empirical estimates of mortality at fewer, carefully chosen concentrations will often serve the same purpose (Denholm *et al.*, 1998).

Table 1. Probit analysis of the response of non-target arthropods to dimethoate.

Species	Sex	No. tested	LC_{50}[1]	95% C.L.[2]	Slope
P. cupreus[3]	♀ + ♂	129	570	380 - 780	3.9
P. cupreus[4]	♀ +♂	218	670	430 - 1100	2.4
P. melanarius	♀	458	1100	730 - 1600*	1.9
P. melanarius	♂	204	500	170 - 730*	2.3
P. madidus	♀	319	570	400 - 690	3.7
P. madidus	♂	141	280	100 - 400*	3.2
N. brevicollis	♀ + ♂	370	640	260 - 1100	1.3
T. ruricola	♂	157	760	690 - 840	7.5

* indicates 90% confidence limits as 95% limits not calculable.
[1] expressed in ppm a.i., sprayed at the equivalent of 200 l/ha. [2] C.L. = confidence limits.
[3] field-caught beetles. [4] laboratory-reared beetles.

Control mortality during these bioassays was negligible, in contrast to results of Cilgi *et al.*(1996) showing high (up to 45%) mortality of untreated subjects to be a major constraint on the estimation of dose-response relationships for carabid beetles by probit analysis. This is probably attributable, in part at least, to differences in bioassay methodology. Cilgi *et al.* (1996) exposed adults of *Agonum dorsale* Pontoppdan, *Demetrias atricapillus* L. and *Bembidion* spp. for 72h without food to insecticide residues on glass plates, whereas in this study, treated individuals were maintained with food on a less artificial substrate. Since there was usually little change in mortality estimates with dimethoate between three and seven days post spraying, the former probably provides a reliable endpoint for this chemical. However, similar bioassays with pyrethroids (unpublished data) have highlighted the importance of longer holding periods over which attention to the well-being of test subjects becomes an essential consideration. The influence of some other biological variables on bioassay results is considered below:

Rearing history

Since the availability of field-caught individuals of non-target organisms is often limited and highly seasonal, risk assessment schemes benefit from exploiting species that can be reared throughout the year in the laboratory. Carabids have historically proved difficult and time-consuming in this respect, although rearing procedures for at least one species, *P. cupreus*, are well established and reliable (U. Heimbach, pers. comm.). One important consideration is therefore whether beetles artificially reared under near-optimal conditions are fully representative of the tolerance of ones likely to be exposed to pesticides in the field. Multiple concentration bioassays comparing laboratory-reared *P. cupreus* with ones collected from the field (e.g. Figure 1) have consistently shown very similar responses to dimethoate, and LC_{50} values from pooling data for several tests do not differ significantly (Table 1). This implies, for *P. cupreus* at least, that laboratory rearing protocols need not cause differences in intrinsic tolerance of insecticides.

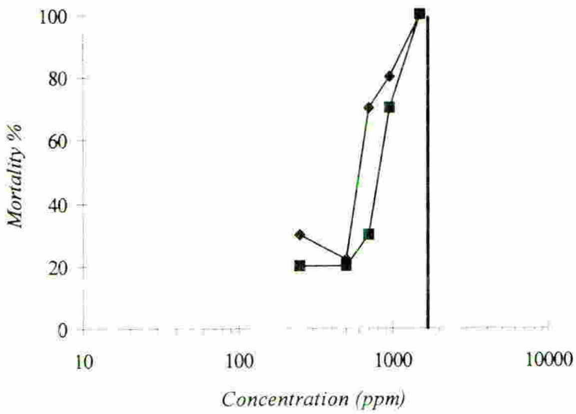

Figure 1. Response of field-caught(◆) and laboratory-reared(■) adults of *Pterostichus cupreus* to dimethoate. Vertical line denotes the recommended application rate for dimethoate to cereal crops.

Does sex matter?

Variation in tolerance between sexes due to differences in size and/or physiology could, if substantial, impose another constraint on the supply of test organisms, since one or both sexes would then need to be evaluated separately. This was investigated with side-by-side bioassays against males and females of both *P. melanarius* and *P. madidus*. Representative data for the latter (Figure 2) exemplify a consistent trend for females to withstand slightly higher concentrations than males, resulting in a barely significant *c.* 2-fold difference in fitted LC_{50} values (Table 1). This demonstrates a statistical advantage to distinguishing between sexes before testing, but also implies that failure to do so will not impair radically the precision or interpretation of bioassay results.

Comparisons between taxa

The choice of appropriate 'indicator' species for risk assessment tests is influenced by several scientific and practical criteria including abundance in arable ecosystems, life-history, and ease of capture and/or laboratory rearing. Likely sensitivity to pesticides is also an important consideration, since if this varies substantially between species within ecologically-functional groups, the ability to extrapolate results from one species to another will be impaired. The present study has shown three species of *Pterostichus* to yield very similar LC_{50} values for dimethoate (Table 1). If this also applies to other chemical classes, these species could in principle be used inter-changeably to contend with regional variation in species composition or differences in phenology. More notably, *N. brevicollis*, which is *c.* one half the body weight of the *Pterostichus* species tested, also showed a comparable response to dimethoate (Figure 3; Table 1). Hence there is no *a priori* basis for equating size with susceptibility within the Carabidae, although further testing of species encompassing a wider range of body sizes is needed to explore this fully.

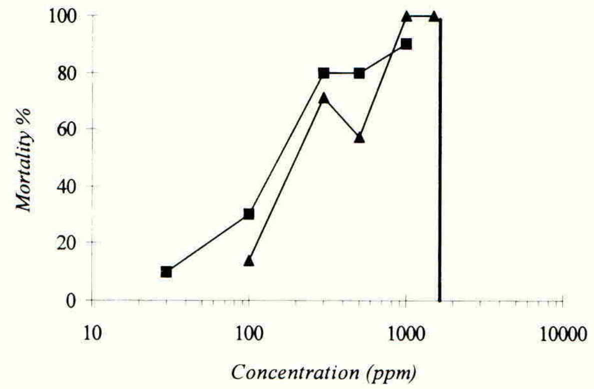

Figure 2. Response of male(■) and female(▲) *Pterostichus madidus* to dimethoate.

Preliminary data for *T. ruricola* also imply little difference between lycosids and carabids in response to dimethoate (Figure 3; Table 1). Testing of other spider species in the families Lycosidae and Linyphyiidae is currently underway.

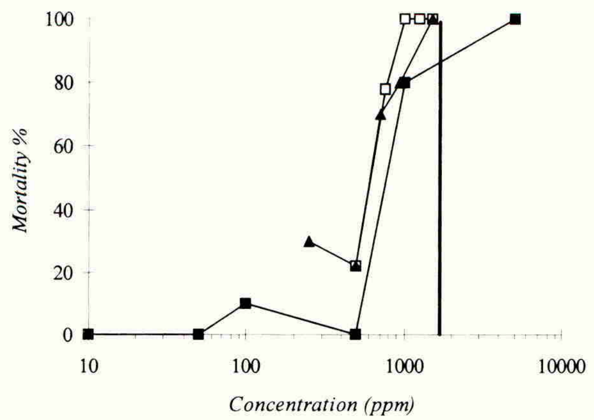

Figure 3. Response of adult *Pterostichus cupreus*(□), *Nebria brevicollis*(■) and *Trochosa ruricola*(▲) to dimethoate. Vertical line denotes the recommended application rate for dimethoate to cereal crops.

CONCLUSIONS

Based on results to date, testing of non-target arthropods at multiple doses is a feasible and worthwhile proposition. The results presented emphasise potential advantages over the usual practice of using only the recommended field application rate. Most bioassays yielded high mortality at concentrations approaching the recommended rate (equivalent to spraying 1700 ppm a.i. under our conditions; cf. Figures 1 and 3), and support a classification, based on this rate alone, of dimethoate as potentially harmful to generalist predators. In most cases, however, mortality declined rapidly at lower concentrations, with fitted LC_{50} values ranging from one half to one quarter of the field rate. Since direct exposure of organisms to insecticide sprays followed by continuous exposure to residues is unquestionably a 'worst case' and probably unrealistic scenario, there is no basis for assuming *a priori* that dimethoate necessarily causes significant mortality of these species in the field. By providing a more accurate measure of the intrinsic toxicity of a chemical relative to its recommended field rate, multiple dose tests are not only potentially more informative than ones conducted at the field rate alone, but also a better complement to subsequent trials under semi-field or field conditions.

Although the need for multiple dose tests appears to be gaining acceptance within Europe, this approach does introduce some logistical and scientific challenges, in particular that of making best use of a possibly limited supply of experimental subjects. This in turn highlights the importance of achieving a consensus regarding the exact objectives of laboratory bioassays against non-target species, and of further research to optimise their design accordingly.

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