Predicting chemical effects on birds: a critical literature review.

Thesis

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Predicting chemical effects on birds:

A critical literature review

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Abstract

The effects of chemicals on birds has created a great deal of public concern and scientific debate. A wealth of information on species distribution, breeding numbers and population changes has provided a good basis for identifying environmental problems after the event. However, the difficulty lies in using such information for predicting possible chemical impacts in the future. Standard laboratory toxicity testing is carried out as a routine requirement for both national and international regulatory authorities, and the prediction of effects is still centred around these tests. Development work has concentrated on test regimes and field validation, but little attention has been focused on the interpretation of the data in the light of the other factors which influence the survival and reproduction of wild birds. Standard and non-standard tests, field trials, monitoring and risk assessment methods and approaches are reviewed. It is concluded that laboratory testing has an important place in the prediction of the effects of chemicals on wild bird populations. However, such tests must be seen merely as a means of screening chemicals for potential effects rather than realistic models of events in the natural world. Test design needs to be varied, and risk assessment flexible, with significant scope for expert judgement. Risk assessment of chemicals should be fate-led to minimize the unnecessary use of laboratory animals by identifying the distribution and availability of chemicals in the environment before testing for effects. Preliminary risk assessment conclusions should be validated in the field. Multidisciplinary studies involving testing, field trials, monitoring and risk assessment should ideally be carried out; disciplines such as toxicology, environmental chemistry, ecology, population dynamics, ethology, physiology and land use geography all have a part to play.

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Contents

1. Introduction

The effects of chemicals, and particularly pesticides, on birds has probably created more public concern and more extensive scientific debate than any other area of pesticide use or pollutant release. Most birds are diurnal which means they are often conspicuous and relatively easy to observe. They are abundant, colourful, inhabit a diverse variety of habitats and overall have great popular appeal. Nowadays, in Britain alone thousands of people watch and study birds. It is not surprising, therefore, that concern is generated about the effects of pollutants on a wide variety of bird species. Much of this concern has centred around pesticides because this group of chemicals are by definition toxic, are applied intentionally to the environment and pose the greatest risk of exposure. In general, the risk from chemicals released from other sources tends to be smaller, but there are several well documented cases of effects being caused by non-pesticidal chemicals. One of the most publicized is that of spent gunshot, and its effects on waterfowl (Anderson, 1975).

The high public profile of birds is continually evident. The first reports of oil spills shown by the media often include the washing on shore of badly oiled seabirds. Ingestion of lead shot and fishing weights causing paralysis and death in swans obtained substantial media coverage. Industrial releases and spills have had some quite dramatic effects on local bird populations, even if these are not manifest on a wider scale or persist long-term. One such incident, involving tetraethyl lead, occurred in the Mersey estuary in the winter of 1979-80, when waders and gulls were found dead along the Lancashire coast with high residues of lead in their tissues (Bull et al., 1983).

In the early 1960s, population declines were recorded in birds of prey, that were later linked with the use of organochlorine insecticides and in particular DDT and dieldrin (Ratcliffe, 1970; Newton, 1979). At the same time, seeds dressed with organomercury fungicides were implicated in cases of primary and secondary poisoning (Borg et al., 1969). Although many of these persistent chemicals have now been banned or severely restricted in use, other chemicals still in use, such as organophosphates and carbamates which are more acutely toxic, often cause direct field kills of birds (Stone, 1979). Moreover some of the organochlorines are still used in large quantities in developing countries. More recently, the problem of secondary poisoning of raptors by 'second generation' rodenticides has been highlighted (Newton et al., 1990b).

A pollutant can be defined as any substance occurring in the environment which at the levels found has deleterious effects on living organisms as compared to 'contaminants' which occur but have no demonstrable effects. Pesticides, because they are biologically active compounds, will all fall under this definition of pollutants. Birds are exposed to most pollutants through their diet, and to a lesser extent via dermal absorption, oral uptake from preening and inhalation. Ground-nesting birds may also have pesticides sprayed directly onto their eggs. Pesticides are by far the largest group of pollutants with demonstrable impacts on bird populations. A very large number of pesticides now exist throughout the world representing a wide range of chemical groups. Some of the main classes of pesticides (chosen on the basis of studies used in this thesis) are summarized in table 1. Different pesticides range from non-persistent and non-toxic groups (to birds) to highly persistent and highly toxic (to birds), and therefore pose greatly differing risks to bird populations.

Table 1: Some of the main classes of pesticide, their persistence in the environment and acute toxicity to birds

Classes of pesticides	Persistence	Bioaccumulation	Acute toxicity to birds	Examples
Inorganic and organometal pesticides				
inorganic metal salts	high[1]	moderate-high[1]	low	mercuric chloride, mercurous chloride
organometals	high[1]	high[1]	moderate	ethyl mercury compounds (includes Ceresan M), methyl mercury compounds, methoxyethyl mercury compounds, phenylmercury acetate
Chlorinated hydrocarbon insecticides				
DDT and its analogs	very high	very high	low	DDT (TDE, DDE; metabolites of DDT), methoxychlor
cyclodienes and related componds	high	very high	high	aldrin, dieldrin, endrin, chlordane
other organochlorines	high	high	moderate-high	toxaphene, chlordecone
Organophosphorus insecticides	low-moderate	low-moderate	predominantly high	acephate[2], azamethiphos, chlorfenvinphos, chlorpyrifos, demeton-S-methyl, diazinon, dimethoate, fenitrothion, fenthion, malathion[2], methamidophos, methyl parathion, monocrotophos, parathion, temephos, triazophos
Carbamate insecticides	moderate	low	predominantly high	aldicarb, carbaryl[3], carbofuran, methiocarb, aminocarb
Pyrethroid insecticides	low	low	very low	cypermethrin, deltamethrin, fenvalerate, permethrin
Chlorophenoxy herbicides	low	very low	low	2,4-D, 2,4,5-T, MCPA
Quarternary nitrogen herbicides	high	low	moderate	paraquat, diquat
Dinitroaniline herbicides	moderate	moderate	very low	trifluralin
Azole fungicides	low	low	low	prochloraz
Anticoagulant rodenticides	moderate	low	moderate-high	warfarin, brodifacoum, difenacoum, chlorophacinone, flocoumafen

[1] - methylation and/or degradation of parent compounds may take place but metals , such as, mercury will persist and bioaccumulate; [2] - moderate toxicity to birds;
[3] - low toxicity to birds. References: The Pesticide Manual (1994); Hayes & Laws (1991); Hudson et al. (1984); Hill & Camardese (1986)

The popularity of birds means that they have been studied in unrivalled detail. Comprehensive records are maintained by a variety of conservation groups, national and international organizations, and research institutes on such aspects as abundance, distribution, breeding success and migration. Long-term trends have been monitored by sampling and observation. Surveys of birds found dead on beaches are done on a regular basis, giving baseline data against which the effects of catastrophic events, such as oil spills, can be measured. Regular and long-term monitoring for pesticides and metals has been carried out from carcasses collected in the field.

It is in using past information for predictive purposes where much of the difficulty lies. Bird populations, especially of small species, have a high annual turnover which means that studying the effects of chemicals is fraught with difficulties, especially when coupled with other variables such as exposure, time of year and species sensitivity. The prediction of possible effects requires an integration of diverse scientific disciplines, including toxicology, ecology, biochemistry, physiology and ethology. The testing methods employed, usually by regulation, need to be carefully assessed. In the case of pesticides, standard tests are often followed by field trials and in some countries by a system of post-registration surveillance. All of this needs to be coupled with continuous monitoring and the ability to reassess implications in light of new evidence.

Birds play an important role in the assessment of the side effects of pesticides and less frequently in the assessment of other chemical contaminants. For many chemicals, they can act as bio-indicators reflecting the likely exposure of the biotic and physical environment as a whole. In the following chapters, both standard and non-standard laboratory testing procedures will be critically reviewed. These tests will be linked to the

field situation through field trials, studies and monitoring. Laboratory and field studies will be discussed in the light of both the hazard and risk assessment procedures which are necessary to regulate chemicals.

2. Standard laboratory testing

Much current prediction of the effects of chemicals on birds rests on the results of laboratory tests. Testing is mostly carried out as a routine requirement of both national and international regulatory authorities. In testing chemicals for the purpose of risk assessment, a tiered system is usually followed, progressing from a simple preliminary protocol to more elaborate systems depending on the requirements of particular situations and the results that emerge.

Major problems with the use of laboratory testing systems include laboratory-to-field extrapolation, inter-test variability, statistical robustness, species and age-related sensitivity and many others. Some of these problems are specific to the laboratory situation, while others apply equally to field testing. Tests need to be realistic with regard to exposure levels and routes of uptake of the chemical but at the same time must not be so complex that statistical robustness is compromised.

Many existing laboratory tests have severe and well documented limitations. However, at the same time field trials are time consuming and expensive. Laboratory studies are carried out under controlled conditions that can give more reliable results but on surrogate species under unrealistic circumstances. Field studies can concentrate on relevant species, but give limited information on subtle effects; large scale field studies are often inconclusive because of uncontrolled confounding variables.

A recent workshop on avian toxicity testing was organized by the Society of Environmental Toxicology and Chemistry (SETAC) and the Organization for Economic

Co-operation and Development (OECD). A pre-workshop questionnaire ascertained the views of prospective participants regarding avian testing. The vast majority of participants agreed that there was a continuing need for guidelines in assessing acute oral toxicity, chronic dietary toxicity, reproductive effects, palatability and acceptance. Approximately two-thirds of the respondents felt that there should be a test for other routes of acute exposure. In general most were not content with the current guidelines for avian testing.

Laboratory tests would appear to be a vital part of the hazard and risk assessment process but exactly what part could they usefully play?

2.1 Acute toxicity tests

Acute toxicity refers to a single administered dose of a material followed by an observation period. The single-dose acute oral toxicity test provides a means of quantifying chemical potency and comparing different substances in a uniform way. Acute toxicity tests are based around the median lethal dose (LD_{50}), a statistically derived single oral dose of a compound which will cause 50% mortality of the test population. A relatively large database now exists on avian acute toxicity of many chemicals (Schafer et al., 1983; Hudson et al., 1984).

Currently three methodologies fit under the rubric of avian acute toxicity testing. In the United States, there are two sets of similar guidelines, one from the US Environmental Protection Agency (EPA) Office of Pesticide Programs and the other from the US EPA Code of Federal Regulations. Both have as an endpoint the LD_{50}. The duration of the tests is 14 days, including an observation period, with a total of 60 birds being used for

each test. The recommended test species are the mallard (*Anas platyrhynchos*) and bobwhite quail (*Colinus virginianus*), using birds of at least 16 weeks old, healthy and of uniform size and weight. Food and water should be offered *ad libitum* with a 15 day acclimatization period and a pre-test fasting period of 15 hours. The administration of the test substance should be via a gelatine capsule although gavage (dose administered by intragastric intubation) is allowed under the Code of Federal Regulations. For each test there should be at least 5 doses with at least 10 birds per dose. The upper limit of the test is set at 2000 mg/kg body weight, ie. if 50% mortality has not been reached at this upper concentration the test is terminated. Individual body weight, mean body weight for each group, total feed consumption for each group, average daily feed consumption for each dose level, and gross necropsy are monitored and parameters such as toxic signs, regurgitation, gross pathology, mortality and recovery are noted. However, whereas the Office of Pesticide Programs states that tests can be run indoors or outdoors, with birds caged by treatment level group and pens large enough to avoid crowding stress, the Code of Federal Regulations dictates that all tests should be indoors with 2 pens of 5 birds, preferably of the same sex per dose level; cage sizes are also stipulated. The Office of Pesticide Programs simply states that temperature and humidity should be controlled if indoors with a photoperiod of 10 hours light and 14 hours dark. The Code of Federal Regulations again stipulates tighter control of temperature (15 to 27°C) and relative humidity (45 to 70%) (OECD, 1996).

The Office of Prevention, Pesticides and Toxic Substances of the US EPA has recently developed test guidelines that attempt to harmonize the guidance and requirements (US EPA, 1995a) . The revised guidelines are probably closest to those of the Code of Federal Regulations.

A draft OECD guideline was also proposed by Germany in 1992. This methodology would use Japanese quail (*Coturnix coturnix japonica*) as the recommended test species, with the endpoints being NOEL (no observed effect level), LOEL (lowest observed effect level) and LLD (lowest lethal dose). The number of birds per test (24) and the acclimatization period (7 days) are less than those stipulated by the US EPA. The number of birds per test is reduced both by reductions in the number of dose levels necessary and the number of birds per dose. Although intensity of light is controlled (65 to 200 lux), the photoperiod is not specified other than greater than 8 hours. The test limit is based on a NOEL of greater than 2000 mg/kg (OECD, 1996).

Much of the argument about the usefulness of the acute oral LD_{50} test has centred around whether it is relevant to the prediction of effects in the natural environment. Protocols now used in testing were originally bioassay methods to determine the relative potency of different substances. Acute tests have been carried out on laboratory mammals, such as the rat, for many years and the results have formed the basis for the classification of short-term hazard of chemicals to humans. It is argued that, with such a large data base, rats could be used to predict the hazard of chemicals to birds. Hudson et al. (1979) compared acute *per oral* (*p.o.*) and 24 hour percutaneous (*p.c.*) LD_{50} values for 21 common pesticides between mallards and rats. Significant positive correlations were found between log *p.o.* and log *p.c.* LD_{50} values in mallards, between log *p.o.* values in mallards and in rats, and between log dermal toxicity index (DTI) values in mallards and in rats. Percutaneous toxicity values were not significantly correlated between mallards and rats. However, Hill (1994) reviewed acute toxicity data on birds (pheasant *Phasianus colchicus* and red-winged blackbird *Agelaius phoenicus*) and rats and found a large difference in

their responses to chemicals of like action, suggesting that reliance on rat data for prediction of hazards to birds is totally unacceptable.

Several studies have been made on aspects of the avian acute toxicity test. Hudson et al. (1972) studied the effect of age on the sensitivity of mallard to 14 pesticides. LD_{50} values were determined for ducks aged 36 hours, 7 days, 30 days and 6 months. The results indicated that young birds were sometimes, but not always, more susceptible to the pesticides tested than were adults. The authors recommended that age-susceptibility factors be considered in the development of standardized protocols. Although age has significant effects on sensitivity, the source of the test birds appears to have little effect. Hill et al. (1984) compared eight stocks of nine-week-old bobwhite for their sensitivity to an acute oral exposure to diazinon. Extraneous variables associated with inter laboratory differences in husbandry were eliminated by incubating eggs and rearing chicks in the same facilities. No significant difference was found between the different stocks. Buerger et al. (1994) compared pen-reared and wild bobwhites and found that there were no differences in the LD_{50} values obtained.

There is great interest in the comparative sensitivities of the three test species: northern bobwhite, mallard and Japanese quail. Romijn et al. (1995) found no significant difference in the sensitivity of northern bobwhite and Japanese quail against a variety of pesticides. Mastrota & Farrar (1994) analysed LD_{50} data and also failed to find any tendency for one species to have generally lower LD_{50} values than the other. However, inter-species comparisons must be treated with caution especially as the two quail species are themselves closely related. Hill (1994) compared seven species and found wide variations in their sensitivity to acute pesticide exposure. Japanese quail were not included

but among other species neither body mass nor taxonomic relationship could be consistently used to predict sensitivity. Baril et al. (1994) recently reviewed inter-species variability in avian acute toxicity testing. They concluded that the best approach was to use a battery of test species for LD_{50} values in order to provide a measure of the sensitivity distribution for each substance. The use of 6-8 species would appear to provide a fair representation. The authors state that, although mammalian toxicology is moving from the use of the LD_{50} test towards a fixed dose protocol, this should be resisted in avian toxicology because of the more limited information on species sensitivity and sub-acute tests. The current guidelines overemphasize the need for an exact LD_{50} value when an approximate value may suffice. If a battery approach is rejected, they suggest that the use of appropriate safety factors must be applied instead. Analysis of acute toxicity data on cholinesterase inhibitors revealed that the relative sensitivity of different test species was reasonably consistent across different chemicals, allowing the application of species specific safety factors and thus the extrapolation from one species to others.

The LD_{50} has become a much misused term. It has often been incorrectly used as a measure of absolute toxicity outside the context of the assay from which it was obtained. It is simply the point of least error on a dose-response curve (Dobson, 1985). The characteristics of a hypothetical dose-response curve are illustrated in figure 1. The typically s-shaped dose response curve derives its shape from the bell-shaped normal distribution curve; the right hand section of the normal distribution is rotated upwards because the vertical axis is a continuous response from 0 to 100%. The point of least error therefore corresponds to the peak of the normal distribution. Hill (1994) pointed out that comparisons and interpretation of acute tests are often focused on the LD_{50}, regardless of its statistical reliability and without reference to the lethality curve or other supplemental

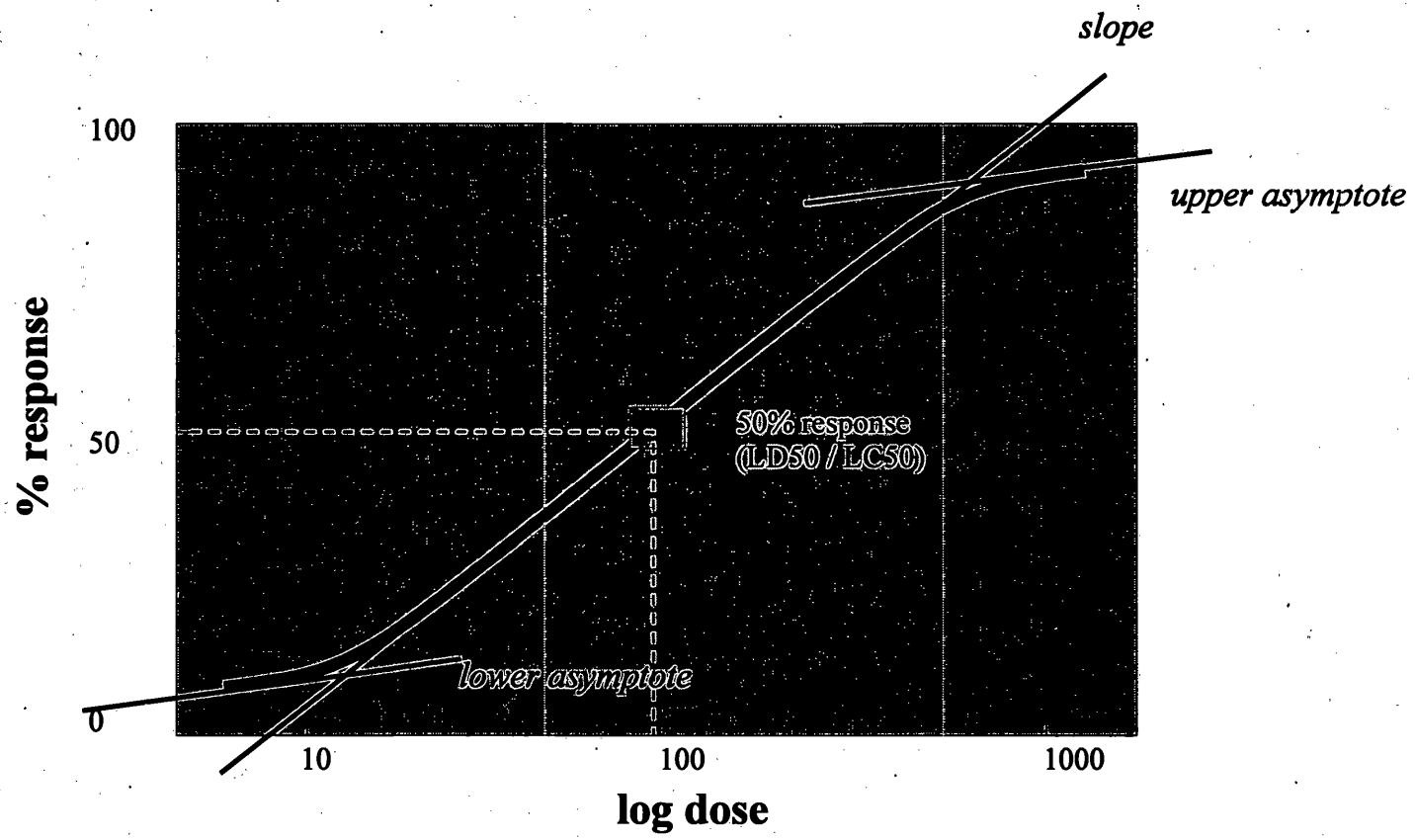

Figure 1: The characteristics of a hypothetical dose-response curve

observations that provide important clues about acute toxicity and hazard evaluation. Dobson (1985) states that two LD_{50} values can give reliable information on the relative potency of test substances only if the other curve parameters, such as upper and lower asymptotes, and slope, are comparable; in practice only if the two curves are parallel in the same assay. If the curves are not parallel, comparisons at LD_{10} and LD_{90} will be quite different from comparisons at LD_{50}.

Another area of uncertainty concerns actual exposure. Hart & Thompson (1995) studied the affect of regurgitation on the oral acute toxicity test. Not surprisingly, the variability of regurgitation caused a serious problem when assessing exposure. The authors suggested that to eliminate the uncertainty caused by regurgitation, consideration should be given to employing a suitably modified short-term dietary test method, rather than using the acute oral LD_{50}, for assessing the risk of dietary exposures to pesticides. Where a non-dietary route of exposure requires assessment, an LD_{50} based on injection might be more appropriate.

At a recent SETAC/OECD Workshop on avian toxicity testing, the working group on acute toxicity agreed that any of the three conventional species could be used for the acute test. It was also agreed, that when there is potential for birds to be exposed to a chemical, the acute oral test was necessary in the first tier of testing. If the chemical substance is not expected to be toxic to birds, then only a single limit test at an upper limit dose would be required. If a toxicological effect was observed, then further testing would be required to determine the dose response curve and LD_{50}. If there is uncertainty in the risk assessment and more information on inter-species differences are considered helpful to reduce this, the Approximate Lethal Dose (ALD) should be determined on further species using an Up

and Down method. The Up and Down test method is used as a range finding test or to evaluate the ALD for a wider spectrum of species (OECD, 1996). Birds are dosed, one dose level at a time, with 2 birds per dose level. If birds survive, the dose level for the next two birds is increased; if they die the dose level is decreased (Bruce 1985). The Working Group recommended that the avian Up and Down test procedure should be submitted to the OECD Test Guideline National Co-ordinators. Insufficient information was felt to be available to develop guidelines for non-oral routes of exposure (OECD, 1996).

In conclusion, findings from avian acute toxicity tests must be treated with caution. LD_{50} values are most useful when information regarding other curve parameters is given and when such parameters can be compared with those from other chemicals of known environmental hazard. Nonetheless, although some criticisms of the LD_{50} test are warranted, it can give a measure of relative toxicity of chemicals to the same test species and relative sensitivity across species, so long as dose-response curve slopes are not radically different. Further development of the LD_{50} as an effective hazard assessment tool requires work in three main areas: studies of the representativeness of test species and inter-species variability in sensitivity, improvements in the quantification of exposure and the development and field validation of extrapolation procedures. Acute tests will remain simple bioassays for lethality and not representative of any real environmental situation or exposure.

Thus, comparative toxicity between chemicals can be estimated from a series of acute tests to a standard guideline. However, a single LD_{50} value is not an absolute definition of the toxicity of a chemical, though it is commonly used this way. Comparative toxicity should

take into account the other characteristics of the curve (slope and asymptotes); chemicals having identical LD_{50} values can have markedly different slopes, reflecting differences in kinetics or mechanism of action. For those substances which are not expected to be toxic, a single limit test at an upper limit dose should be carried out. The need to restrict the number of birds used within the testing regimes must always be of prime concern as long as this does not compromise the statistical robustness of the tests; both the single limit dose and the Approximate Lethal Dose have the advantage of using fewer test animals.

2.2 Dietary toxicity tests

Avian dietary toxicity tests determine the median lethal concentration (LC_{50}) of a chemical defined as the quantity of toxicant in the diet calculated to kill 50% of the test population within a defined period. The LC_{50} value is expressed as mg/kg diet. The subacute dietary toxicity of chemicals to birds has been estimated under a standardized protocol at the Patuxent Wildlife Research Center, Maryland, USA, since 1965. The US Department of the Interior have summarized their dietary LC_{50} tests (Hill et al., 1975; Hill & Camardese, 1986).

There are currently three recognized dietary toxicity tests following a similar format: the US EPA Office of Pesticide Programs avian dietary LC_{50} test (No. 71-2; 1982), the US EPA Code of Federal Regulations avian dietary toxicity test (No. 797.2050; 1993) and the OECD avian dietary test (OECD, 1984a). There is now a proposal from the U.S. Office of Prevention, Pesticides and Toxic Substances to harmonize the tests (US EPA, 1995b). All three tests are based around a 5 day exposure followed by 3 days on a 'clean' diet. Two of the tests allow longer observation periods (up to 16 to 18 days if necessary). The main

endpoint is an LC_{50} although the OECD test includes others such as NOEC (no observed effect concentration) and LC_{100}. The total number of birds for each test ranges from 60 to 70, with the age of the birds ranging from 10 to 17 days, because young birds tend to consume more food per body weight than older birds. The US EPA test species are the mallard and northern bobwhite; however, the OECD test allows the use of other species such as Japanese quail, ring-necked pheasant, red-legged partridge (*Alectoris rufa*) and pigeon (*Columba livia*). The OECD test stipulates a photoperiod of 12 to 16 hours; the US EPA prefer a diurnal photoperiod, but 24 hours of light is deemed acceptable. All three tests suggest an indoor trial, although the Office of Pesticide Programs also allows the test to be outdoors. The size of the caging is stipulated in all cases, with *ad lib* food and water required throughout the test period. The upper limit of the test is set at an LC_{50} (US EPA) or a NOEL (OECD) of greater than 5000 mg/kg feed (dry weight). The parameters to be examined include toxic signs such as convulsions and lethargy, abnormal behaviour and mortality. Mean body weights and food consumption per pen should be monitored. The US EPA tests also suggest that gross necropsy and pathology should be examined (OECD, 1996).

The avian dietary toxicity test has now been in use for over thirty years, during which much investigation has been carried out on the details of the test. Several studies have concentrated on the variability of sensitivity both within and between species. Hill (1981) studied the variability in toxic response of the Japanese quail to the dietary toxicity test. The basic protocol was found to yield good within-laboratory reproducibility. The LC_{50} values increased with age, with the change being reasonably predictable from 7 to 21 days of age. The author concluded that it is essential that time-related response patterns, such as food consumption, onset and remission of overt toxicity and mortality, are closely

monitored in order to evaluate the potential hazard. He recommended that testing should always be carried out on birds of the same age to optimize comparisons. Hill & Camardese (1986) reviewed the dietary toxicity test for 193 environmental contaminants, including pesticides, organic solvents and adjuvants (permitted non-pesticide products added to pesticides). They found good inter-test reproducibility of response with Japanese quail. However, they pointed out that the subacute toxicity varied widely among structurally similar chemicals and between different formulations of the same chemical. Hence, conclusions about the lethal hazard should be made cautiously until the actual formulation of interest is tested. Hill et al. (1977) tested the dietary toxicity of dieldrin repeatedly during 8 years of testing on young bobwhites, Japanese quail, ring-necked pheasant and mallards. Toxicity (5 day LC_{50}) was estimated at least 18 times for each species over 6 to 18 generations. No time-related changes were detected in LC_{50} values for any of the species.

Hill et al. (1975) compiled the results of nearly 10 years of dietary toxicity testing. Inter-species comparisons revealed that the overall susceptibility was size-related: quail > pheasant > mallard. The order of susceptibility varied but a characteristic order usually prevailed within a given class of chemicals. The results suggested that, regardless of test species, the relative toxicities of different chemicals in the same class would be similar if the testing conditions remained constant. However, unpredictable differences in species' susceptibility were found often enough for tests of at least two species to be desirable. Romijn et al. (1995) concluded that, if any differences existed between bobwhite and Japanese quail in their relative sensitivities in the dietary toxicity test, they were likely to be small and insignificant. However, Mastrota & Farrar (1994) found a significant difference in sensitivity between the two species. Both the t-test and the sign test indicated

a statistically significant tendency for the LC_{50} of the bobwhite to be less than that of the Japanese quail for each of the chemicals tested. The bobwhite appeared to be 1.46 times more sensitive to a range of chemicals than the Japanese quail based on the data set analysed. Mineau et al. (1994a) carried out inter-species comparisons between mallard and bobwhite quail with regard to the LC_{50} test. The results suggested that the LC_{50} values obtained for one species could not be used to predict those of the other. It should be noted that the same inter species comparison for the acute LD_{50} test revealed a significant correlation between the sensitivity of mallard and bobwhites. The authors therefore concluded that extrapolation from the LC_{50} test on any one of the test species to other avian species was unreliable.

Calculation of the LC_{50} and its confidence limits is based on the assumption that birds exposed to certain dietary concentrations of a test chemical will consume the same amount of food in each treatment group. However, the assumed daily food intake (DFI) can be quite different from the measured DFI. The assumed and measured daily chemical intake will be directly related to the assumed and measured DFI. Reductions in food consumption can be induced by a variety of factors such as taste repellency, aversive conditioners and anorexia conditioners (Luttik, 1993). In LC_{50} tests with bobwhite exposed to parathion, the measured DFI was 7.2 g/bird/day in control birds and decreased to 1.5 g/bird/day at 251 mg/kg food (Bennett, 1989a). The decrease occurred steadily through each of the 6 dose levels. Luttik (1993) concluded that, based on the data analysed and in tests in which food consumption was decreased, the LC_{50} should be considered as a measure of the relative vulnerability of a species to a dietary exposure to a toxicant and not as a measure of sensitivity. Mineau et al. (1994a) stated that, for an LC_{50} to be an unbiased measure of the inherent dietary toxicity of a compound, the food

consumption at each exposure concentration must be the same. In tests in which food consumption decreases with increasing exposure concentration, the LC_{50} test becomes more a measure of the bird's ability to survive periods of drastic food reduction than a measure of a product's inherent toxicity. The calorific and nutritional quality of the feed used in dietary tests is seldom measured; nor is the water content. In other words, neither the chemical nor the biological quality of the food is usually analysed. However, the type of food is likely to have a significant impact on the dietary toxicity of a chemical. In particular, the higher the nutritional value of a particular food and the lower its moisture content, the less the weight of food required, thereby reducing the intake of toxicant (Mineau et al., 1994a). Kenaga (1973) recognized that, for any given species, the daily rate of food consumption was directly related to the moisture content, and the calorific and nutritional value of the food.

Problems with the determination of the dietary LC_{50}, relate primarily to the test conditions employed. Mineau et al. (1994a) suggest that a more useful predictor of risk might be a risk index based on the ratio of predicted environmental concentration (PEC) to LC_{50}. However, for such a calculated risk index to be predictive, it is essential that the LC_{50} ratios between chemicals must be consistent between species and this was found not to be the case. The authors concluded that the dietary LC_{50} value should not be used as a trigger for higher tiered studies. The only area where the LC_{50} might offer some insight would be that of chronic toxicity. Comparing a prolonged dietary or repeat-dosing exposure to a single acute dose could provide an indication of a pesticide's potential to cause chronic or cumulative toxicity (Kenaga, 1973). The Working Group on dietary toxicity testing at a recent SETAC/OECD Workshop on avian toxicity testing recommended that the test duration should be increased from 5 to 21 days followed by a recovery period of 7 days

(OECD, 1996). Mineau et al. (1994a) suggest that it might be possible to improve the test by considering the dietary dose at which the first mortalities are recorded or by restricting the analysis of mortality to those deaths resulting from intoxication rather than food deprivation.

At first sight, the dietary toxicity test seems more 'environmentally relevant' than the acute test. The dietary route of exposure seems to relate more to the field situation than a single oral dose. However, the dietary test is still a simple bioassay for lethality and as such should be treated with caution. The dietary test offers some insight into the chronic or cumulative toxicity of chemicals and increasing the length of the test from 5 to 21 days might improve this. For chemicals that are repellent or cause reduced food intake, the test may be more a measure of effects of starvation rather than of direct toxicity. Unlike the acute LD_{50} test, extrapolation from the LC_{50} test on any one of the test species to other avian species appears to be much less reliable. Comparative toxicity between a range of dietary LC_{50} values across species or chemicals is more complex than for acute LD_{50} values. Differences in result may derive from time differences in onset of toxicity or differences in food eaten, and therefore dose received. Comparisons should, therefore, be made between calculated LC_{50} values based on reported body weights and food consumption in the actual tests. For valid comparisons of LC_{50} values food consumption (= chemical intake) needs to have been measured or estimated as precisely as possible.

2.3 Reproduction tests

Concern has centred on the sublethal effects of chemicals on birds and in particular those affecting reproduction. Acute and dietary toxicity tests were not seen as using 'real'

ecological endpoints. Therefore, the detection of effects on reproduction was perceived as a high priority for the regulatory bodies. A protocol was first developed by the US EPA in 1975 and further refined in 1978 and 1982.

There are currently three recognized test guidelines based on reproduction and a proposal from Germany for an avian subchronic test which includes effects on reproduction. The three recognized test guidelines are the US EPA Office of Pesticide Programs avian reproduction test (No. 71.4; 1982), the US EPA Code of Federal Regulations reproduction test (No. 797.2130 for bobwhite & No. 797.2150 for mallard; 1993) and the OECD avian reproduction test (OECD, 1984b). There is now a proposal to harmonize the tests (US EPA, 1995c). All three tests permit the use of bobwhite or mallard, but the OECD test also allows Japanese quail. The test substance is mixed with the diet, which is fed *ad libitum* throughout the test period. Confirmation of substance concentration in the diet (dry weight) is required. Unstable or volatile compounds are specifically excluded from use in the guidelines. Exposure to the compound is for 8 weeks under non-stimulatory lighting conditions (short days of 7 to 8 hours of light per day) followed by initiation of breeding using long days (16 to 18 hours of light per day; increased to 19 to 20 hours in US EPA tests) and further exposure for 8 to 10 weeks during egg laying. This makes a total time period for the test of approximately 20 weeks. The housing is indoors, except that the OECD test also allows the use of outdoor pens. The sex ratio of caged groups varies depending on the species used, with 1 male to 2 females for quail and 1 male to 3 females for mallard (OECD, 1996). A stipulated variety of reproductive parameters are measured during the test, including egg production, eggshell thickness, percentage of cracked eggs, viability of embryos, hatchability of eggs and survival of hatchlings (maintained on a 'clean' diet) to 14 days. Normal values for the various reproductive

endpoints are provided as guidance. The other parameters that have to be measured and recorded during the reproduction test are: i) mortality and signs of toxicity, ii) body weights of adults, iii) body weights of young, iv) food consumption of adults, v) food consumption of young, and vi) gross pathological examination of adults at termination.

Recently there has been a proposal from Germany for an avian subchronic toxicity test including effects on reproduction in the Japanese quail. It is a much shorter test than the other reproduction tests with a total duration of 10 - 11 weeks. The exposure period is even shorter, being the 6 weeks prior to the study of reproductive effects (OECD, 1996). Schlatterer et al. (1993) reports that the six week test was performed by five laboratories studying the effects of tributyltin oxide on Japanese quail. They found that the results for most of the test parameters were consistent between laboratories.

There has been more discussion of the relevance of the reproduction test to the environmental situation than any other test guideline. This is probably because it was originally designed to try to answer some of the criticisms of the acute and dietary lethality tests. However, there are now concerns about the continuing relevance of the test. It was designed principally to detect eggshell thinning and other impacts resulting from the bioaccumulation of chlorinated hydrocarbon insecticides.

Dobson (1992) assessed the relevance of the OECD avian reproduction test, although similar arguments can be used with the US EPA tests. The guidelines were discussed in the light of the current knowledge regarding avian physiology and photoperiodic response. It was concluded that there is justification in retaining the current test species because they are easy to keep and fundamentally their photoperiodic response is the same as other

species. However, it was suggested that marginal photoperiods should be used, approximating to those likely to be experienced in making a physiological 'decision' at the onset of breeding. The populations of birds used in the tests should be homogeneous in their photoperiodic response. The effects of chemicals on egg laying would be better conducted for short periods on birds already in regular lay. In fact, for non-persistent chemicals, a short test of 4 weeks covering the onset of reproduction would probably suffice. It is important that food consumption is monitored, because not only does this directly affect exposure levels, but it can also influence the egg-laying and other physiological responses assumed by the test. A choice between dosed and undosed food either in the main test or in a secondary one would allow results to be extrapolated from hazard to risk assessment more easily. Natural incubation might profitably be included in a future protocol. Finally, it was pointed out that nesting behaviour is not currently part of the test and this has a large influence on reproductive success in nature.

In order to assess the reproduction test, Mineau et al. (1994b) reviewed 134 studies submitted by industry on 69 different pesticides. An attempt was made to determine whether the test could identify pesticides that have a known potential to affect reproduction in wild birds. The authors used a clustering procedure to assign measured variables to parental, developmental and eggshell effects. This was identical for the two bird species tested (northern bobwhite and mallard). Nineteen of the 69 pesticides tested were found to cause developmental effects at levels lower than those causing parental toxicity. Little similarity was found in the effects of pesticides on the two bird species used, casting doubts on the ability of the test to extend the results to other species. Several general considerations emerged from the review which are important for future developments of the reproduction test. There needs to be a better justification and careful

consideration of the dose levels chosen for any reproduction study. Species tend to vary in their reproductive response to any particular chemical exposure, so it is insufficient to test a single species in order to generalize. On a case-by-case basis, biochemical or histological procedures should be implemented to explain and clarify some of the results obtained. An effort should be made to maximize the information that can be gained by the test. An important area that needs to be addressed at some future stage is that of reproductive behaviour. The authors found no evidence that the avian reproduction test has triggered a field study to monitor reproduction in wild bird species. It was suggested that some form of field verification should be attempted, looking at exposure and reproduction in a realistic field context.

Bennett & Ganio (1991) reviewed the US EPA avian reproduction test and concluded that sources of variation unrelated to the pesticide treatment should be identified and reduced. They suggested that acceptability guidelines for egg cracking, eggshell thickness and percent fertility, hatching and survival should be set, based on historical control data. It is important that tests are designed to have sufficient statistical power to detect effects. It was pointed out that some pesticides which do not satisfy the existing criteria for initiating the reproduction test might affect reproduction from short-term exposure. Test methods for measuring reproductive effects from short-term exposures need to be standardized. Gile & Meyers (1986) carried out the avian reproduction test using 7 and 11 month old mallard. There was a significant effect of adult age on the weight of ducklings at 14 days. However, some of the hens produced phenotypically-different ducklings suggesting the presence of a different genotype and this may have influenced the subsequent difference in duckling weight. The authors concluded that, whilst overall the difference in ages of the

two sets of mallard did not appear to affect the test significantly, it did demonstrate the importance of using phenotypically and genotypically similar test birds.

There is a great temptation, when designing a test to predict environmental hazards, to include too many variables. The result is a test which cannot produce statistically meaningful data. Mineau et al. (1994b) concluded that the highest dose level tested should be the level that approaches lethality and that the test should provide information to help design an eventual field trial or monitoring exercise. The test should be seen as a means to identify mechanisms of toxic action and not as a realistic simulation of the reproductive process of birds exposed to a specific pesticide use pattern. Therefore, decisions regarding the test duration should be made on the grounds of increased statistical power rather than increased realism.

The ability of several statistical tests to detect effects on egg laying, eggshell cracking and hatching rate were investigated by Collins (1994). He concluded that the number of cages specified in the guidelines is insufficient but varied for each of the three variables studied, being highest for the detection of effects on egg laying. Increasing the test from 8 to 12 weeks reduces the power of the test to measure hatching effects. Williams' test was generally the most efficient of the tests considered. In the mallard test, the adoption of a 1:1 sex ratio may result in a loss of power compared with a 2 males to 5 females design. MacLeod (1994) reviewed the statistics used in approximately 100 avian reproduction tests submitted. Various statistical methods were used, many of which were inadequate and reduced the ability of the experiments to assess effects. Several reasons were identified, including a multiplicity of variables and methods (current methods are too general), unclear objectives and lack of information on current protocols. The main

objective of the report was to identify the best procedures and statistical methods for the reproduction test. For maximum efficiency, all statistical tests should be one-tailed, checking specifically for the effect most likely to occur. Another objective was an examination of data quality; some of the factors affecting quality were identified as mortality, disease and inconsistency in the birds' reproductive capabilities.

In the lead up to a recent SETAC/OECD Workshop on avian toxicity testing, participants responded to a question on the value of the avian reproduction test. The most important roles identified were that the test should reveal the potential of a substance to affect the reproductive system of birds, that it should simulate a chronic exposure situation, and that it should define a maximum tolerated concentration for birds. Some of the respondents favoured other role statements: these were to simulate avian reproduction under realistic conditions of exposure or to act as a possible trigger for field studies and help in their design (OECD, 1996). The lack of a clear consensus on the role of the test indicated a need for discussions regarding the basic premises on which the reproduction test is based.

The avian reproduction subgroup of the above workshop discussed the current reproduction test guidelines in detail and were able to reach a consensus in many areas (OECD, 1996). They state that the test should evaluate the effect of a test chemical on avian reproductive performance and provide a mechanism, when data are combined with exposure information, for predicting effects on wild birds. The existing basic test of reproduction in birds is suitable for all chemicals; the test should be used for all pesticides and also for major chemicals which show persistence or a high potential to bioaccumulate. The test should be at the first tier of product testing, forming part of the standard information package for all pesticides proposed for registration. It was agreed that the

Japanese quail was the best species to use as the main test species, followed by the mallard if a second test was required. The group agreed that the use of uncertainty factors (numerical factors which reflect the degree of confidence in the dataset) is appropriate in the context of the reproductive endpoints and that their use might eliminate the need to test several species. Therefore, the test would comprise a first tier test with Japanese quail, with a choice of a second species or the application of uncertainty factors to take into account species sensitivity. The workgroup recommended that dose levels be set so as to reveal significant effects on reproductive output at least at the highest dose. An upper limit of 1000 mg/kg diet, or twice the predicted environmental concentration (PEC), should be set. To improve the statistical power of the test, it was agreed that the pre-laying dose period should be eliminated, whilst using proven breeders only with the pre-dosing data as a covariate. No observed effect concentrations (NOEC) should continue to be used, but reported alongside the statistical power of the test from which they were derived. The use of lowest observed effect levels to replace the NOEC should be investigated.

The subgroup commented on the test procedure details and made the following recommendations: (1) Tests should allow for the collection of pre-dose data for a minimum of 2 weeks after the peak egg production; (2) At least 6 weeks of dosing should follow during which all eggs are collected and set; and (3) Only minor modifications need to be made to the current set of endpoints:

i) eggshell breaking strength and the standard eggshell thickness to be measured.

ii) differentiation between infertility and early embryo death at first candling (6-11 days of incubation) to be made.

iii) gross examination of chick gonads and sex determination of chicks at the highest dose level, with examination of other groups when effects are found.

iv) body weight to be determined just before initiation of dosing and at the end of the study.

v) clinical signs of toxicosis, such as lethargy, depression, wing droop, and ruffled feathers, to be recorded at least once each day.

vi) and, at the termination of the study:

- necropsy and assessment of gross pathology to be performed
- wet weight of liver, spleen and male gonad to be determined
- histopathology on organs showing gross pathological changes.

Other tests for avian reproduction were also identified by the subgroup, including surface exposure of eggs with chemicals and a short exposure test for pesticides applied at high levels but rapidly degraded in the environment. A discussion of other studies on reproduction including these considerations will be given in chapter 3.

The consensus of the subgroup was for a new avian reproduction test to be drafted and submitted to OECD for consideration. Several areas of future research were identified, such as investigating the relative sensitivity of the test species, the use of biomarkers, refinement of the statistical analysis, development of a short term exposure test, development of a full breeding cycle test in a passerine species, and field validation of the test.

Overall the reproduction test appears to be the most 'sophisticated' of the standard tests; however, it implicitly assumes a very simple model for the control of reproduction. The

test has several problems but, with some modifications, it can provide a good indication of the direct effects of chemicals on reproductive physiology. However, even with major modifications the test will tend to be a poor indicator of population effects in the field because both test species and conditions are inappropriate. The reproduction test is, therefore, useful for indicating potential sublethal effects of chemicals, but does not realistically simulate the field situation. A recommendation for using the full breeding cycle of passerine species has been put forward as a possibility for improving the predictive value of the set of tests for birds. The use of passerine species which feed their young (atricial) would at least be more representative of the vast majority of bird species, unlike the current test species which all have young who feed themselves (precocial). Passerines will not, however, breed in captivity following regular handling (as current test species will). A new test would have to take the format of a population left to breed over a set time with simple monitoring of hatching and survival of young at the end. It remains to be seen whether this will be incorporated into the testing regime, and, even if it is, to what extent it will improve the ability of the test to mimic the field situation.

2.4 Acceptance/palatability tests

A method for testing the rodent repellency of chemicals on packaging material was developed in the early 1960s. The method was based on the concentration of a chemical required to repel 50% of the test rodents (R_{50}). The method was later adapted for birds (Starr et al., 1964), and subsequently modified to allow more rapid and effective appraisals of potential avian repellents (Schafer & Brunton, 1971). Schafer et al. (1983) used the R_{50} to generate a repellency-toxicity index or hazard factor. Using knowledge of body mass

and food consumption, it gave an estimate of the amount of chemical that could be ingested at the R_{50} level. Such a value, when divided by the acute oral LD_{50}, provided an index for assessing the likelihood of acute oral poisoning in the wild. There are now several acceptance/palatability tests that have been developed for birds: BBA 25-1 test, INRA test and median food avoidance tests.

The acceptance test for use on baits, granules and treated seeds was developed in Germany (BBA 25-1). It is a 15 to 17 day test using Japanese quail, pigeon and ring-necked pheasant. Both sparrows and partridges are named as other possible species. The test consists of a sixteen hour fast period, followed by an 8 hour exposure with the cycle being repeated once for quail and three times for pigeons and pheasants. The test substance is mixed with bird feed and the birds are exposed to seed and/or bird feed mixture which is scattered on the floor of the cage. Initially a rigorous test is carried out, feeding a mixture of 75% test substance (by weight) and 25% standard food. If mortalities occur, then a repeated test of 10% test substance and 90% standard food is performed. General observations for symptoms of poisoning, mortality and unusual occurrences are made throughout the test. Body weights are measured before and after the test. Macroscopic and pathological examinations are carried out, especially on the crop and gizzard of dead birds (OECD, 1996).

The INRA (France) method is a test of the acceptance of feed and seeds treated with a repellent, by captive birds. The test duration is specified as at least one day and uses both the grey partridge (*Perdix perdix*) and the red-legged partridge. It is a simple choice test comprising a no-choice test with 100% treated food and a two-choice test of 50% treated food in one cup and 50% untreated food in the other. The positions of the cups are

alternated. The no-choice test is conducted first, followed by the two-choice test if birds continue to eat in the no-choice test and especially if there are mortalities. Feed consumption is monitored throughout the test (OECD, 1996).

Another protocol for the evaluation of repellent effectiveness on birds was developed at the Denver Wildlife Center, USA. It is a 5-day test, permissable on a wide variety of bird species, usually red-winged blackbird, European starling (*Sturnus vulgaris*), Canada goose (*Branta canadensis*) and mallard, with the addition of house finch (*Carpodacus mexicanus*) and American robin (*Turdus migratorius*) on fruit. The test begins with a two-choice screening test, followed by a no-choice test if desirable on grounds of potential cost/benefits or chemical effectiveness. The two-choice test is repeated more rigorously after the no-choice test if the consumption and preference ratio from the screening test is less than 0.34. The test is carried out over 5 successive fasting and exposure cycles, lasting 2 and 4-6 hours respectively. Body weight, feed consumption, symptoms of malaise and preference ratios are monitored (OECD, 1996). Mean total consumption of feed, mean preference ratios, effective avoidance index (EAI) and the median food avoidance concentration (FAC_{50}) are all measured. The FAC_{50} is the maximum concentration of a test compound that is expected to result in equal consumption of treated and untreated feed by a group of birds given free access to both. It is thus the highest expected dietary concentration of toxicant that will not result in detectable avoidance behaviour by an exposed population. The EAI can be defined as the ratio of LC_{50}/FAC_{50} and is a measure of the margin of safety (Kononen et al., 1986).

Bennett (1989a,b) used another endpoint; namely the dietary concentration above which birds discriminated between feeders by consuming a greater proportion of untreated food,

defined as the discrimination threshold (DT). Unlike the Denver Wildlife Research Center test, those by Kononen et al. and Bennett use juvenile bobwhite quail as the test species. There is no starvation period before the test. In the test of Kononen et al., a choice of 50% treated food and 50% untreated food is used. The same choice test is used by Bennett (1989a,b), but another test of 90% treated and 10% untreated feed is also made. Both tests monitor mortality, toxic signs, behaviour, body weight and food consumption.

The FAC_{50} is calculated in the same manner as the dietary LC_{50}, i.e. by probit analysis using the response variable as the proportion of total food that was treated (Kononen, 1988). The DT values are calculated as the intersection of a two-phase regression analysis, with log concentration of treated food as the independent variable and the log of the ratio of untreated to treated food consumption as the dependent variable (Bennett & Schafer, 1988).

Luttik (1993) reviewed avian food avoidance behaviour and concluded that small alterations of the standard dietary LC_{50} test could make other repellency tests with birds superfluous because the standard LC_{50} test provides enough information about the repellent properties of a substance. Repellency tests can be used to provide information about edibility of the granules and seeds which results in less suffering of test animals. It is recommended by the authors that measurement of food consumption would provide information about the learning abilities of the species tested. In other words, it reveals when food avoidance appears. A No Repellent Concentration (NoRC) of a substance, the concentration in the treated food at which the birds in the test will eat the same amount of treated food as the untreated food eaten by the control group, can be calculated. However,

Mineau et al. (1994a) concluded that, on the basis of the limited field evidence available, the food avoidance levels obtained from LC_{50} studies have little or no predictive value.

Prior to a recent workshop on avian toxicity testing, participants were asked by questionnaire whether standard guidelines are required for food avoidance or whether the information can be obtained from the food intake/body weight data of a standard dietary toxicity test. The collective response was that, although the dietary toxicity test yields useful information on food avoidance, it is not sufficient to make a proper assessment. Some of the shortfalls identified include insufficient information on palatability and on why food avoidance occurs, and qualitative rather than quantitative information on avoidance (OECD, 1996). Whether avoidance testing can be based on information from a modified dietary toxicity test, or whether specific tests can be produced from the existing palatability/acceptance tests, there appears to be a clear need for more harmonized guidelines.

The Working Group on avoidance testing at the above workshop concluded that many of the aspects of the methodology proposed for avoidance testing require refinement. They recommended a screening test as a simple and sensitive measure of avoidance to decide whether a chemical has potential as a repellent. Given an appropriate result, this could be followed by a realistic and severe test which produces results specific to a set of clearly defined experimental conditions (OECD, 1996).

3. Non-standard testing

3.1 Behavioural effects

The behaviour of an organism represents the final integrated result of a diversity of biochemical and physiological processes (Warner et al., 1966). Behaviour patterns are known to be highly sensitive to changes in the state of an organism. This sensitivity is one of the key values for their use in exploring sublethal toxicity. Such clinical behavioural signs as lethargy and tremors are now measured qualitatively in standard testing procedures, such as response to food treated with contaminants. In this subsection, I shall attempt to explore the effects of contaminants on behaviour patterns rather than on symptoms of poisoning. This is an aspect on which there are no standard procedures.

Peakall (1985) identified three main categories of frequently-used behavioural tests, involving (i) operant and visual cliff experiments; (ii) approach and avoidance; and (iii) courtship and nest attentiveness. The first category explores the ability of a bird to learn and re-learn certain behavioural actions, whereas the other two make use of essentially natural behaviour.

Operant behaviour and visual cliff experiments cannot be directly related to survival prospects, but it is assumed that a decrease in learning ability is unfavourable. Such tests appear to be very sensitive to toxicant exposure. Most operant conditioning tests are based around those developed by B.F. Skinner in the 1940s. Birds are trained to respond to a lighted key to obtain food, enabling the learning skills of treated and untreated individuals to be compared.

Armstrong et al. (1963) trained food-deprived pigeons to peck on a key to obtain food to receive a reward. Birds were trained to respond to either food provided after a certain number of pecks (fixed ratio portions), or to a food reward after a certain time interval had elapsed (fixed interval portions). Exposure to mercury vapour (17 mg/m^3) reduced the average rate of response, but birds recovered after mercury exposure ended. At mercury concentrations of 0.1 mg/m^3, there was no significant effect on operant behaviour (Beliles et al., 1967). Barthalmus et al. (1977) used a similar system and found that daily doses of 12.5 mg lead acetate/kg body weight by gastric intubation decreased the rate of response to both fixed ratio and fixed interval schedules. Gesell et al. (1979) trained adult bobwhite quail to peck a green lighted key to receive food. Birds were exposed to dieldrin (50 to 300 µg administered in 0.5 ml of corn oil every other day) for 28 days. Significantly slower and less accurate responses were observed at dieldrin concentrations of 100 µg or more during the exposure period compared with the 14 day pre-exposure period. Significant changes in operant behaviour were detected in all bobwhites that had at least 5.7 mg/kg (wet weight) of dieldrin in their brain tissue.

Kreitzer (1980) developed a methodology based on operant conditioning to measure the effects of environmental contaminants on the behaviour of adult bobwhite quail. In this procedure, birds are maintained at 85% to 90% of normal body weight and are, therefore, motivated to peck at lighted translucent keys in the operant conditioning box for a food reward. The test box is a sound-attenuated chamber with two pecking keys and a feeder. The quail are trained to peck a lighted key for food and are tested for their ability to distinguish between two lighted patterns such as vertical black stripes versus horizontal black stripes. The initial test during which the bird learns that one pattern brings a reward is the acquisition test. When the reward is switched to another pattern, the learning period

is called the reversal test. Exposure to toxaphene (10 mg/kg diet) or endrin (0.1 mg/kg diet) for 138 days significantly increased the number of errors committed by the birds. Bunck et al. (1986) found no effect on discrimination learning in bobwhite with paraquat in the diet at concentrations up to 100 mg/kg for 60 days.

Visual cliff experiments involve the choice between visually shallow or deep sides of a box with a glass floor (Tallarico & Farrell, 1964). Baxter et al. (1969) tested the behaviour of pheasant chicks on a visual cliff. They found that those chicks hatched from eggs laid by hens exposed to dieldrin chose the deep side, whereas those from the control group chose the shallow side. Similar findings were reported by Dahlgren & Linder (1971). Significantly more pheasant chicks from eggs laid by hens exposed to PCB chose the visually deep side or made no choice of sides compared with control birds.

Whilst both operant behaviour and visual cliff experiments test a birds' ability to learn, and it is assumed that a decrease in learning ability is unfavourable in the environment, it is extremely difficult to extrapolate the results from such tests to the field situation. The tests appear to be sensitive but their value is probably limited to the comparative toxicity of chemicals.

Approach and avoidance behaviours are more directly related to survival, but the results of such tests are generally unquantified. In the field, maternal calls to newly hatched ducklings are extremely important in leading the ducklings away from the nest site. Heinz et al. (1979) report that, at the Patuxent Wildlife Research Center, the approach behaviour of ducklings to maternal calls was tested in an experimental runway. The test system comprised 10 identical runways 62 cm in length, with a loudspeaker at one end.

Underneath the speaker was a sensitive treadle which detected the presence of a duckling. The time taken for a bird to complete the distance was recorded, as well as any subsequent behaviour. Heinz (1979) found that ducklings from parents fed 0.5 mg mercury/kg diet, as methylmercury, for three generations were less responsive to maternal calls than were control birds. However, no significant effect had been observed by Heinz (1976b) during second generation studies. Martin et al. (1991) used a similar experimental set-up to study the approach response of ducklings which had been led through carbofuran-sprayed vegetation for varying distances. Four identical 184 cm runways were used and each was lined with white acoustical tiles. The approach response behaviour became significantly slower with increasing spray rate and exposure distance.

A second behavioural test measured the avoidance of a frightening stimulus. In the wild, it is important that young birds are able to escape from danger in order to survive, and one of the ways of escaping is to flee. To test such behaviour, researchers at the Patuxent Wildlife Research Center used a moving silhouette (black ellipse) tapped lightly against the end of the pen. Kreitzer & Heinz (1974) found that the group avoidance response of Japanese quail chicks was significantly suppressed by exposure to chlordane, dieldrin, endrin, Ceresan M and Aroclor 1254, but not by DDE. In more recent studies at the same Research Center, the fright stimulus consisted of a 5 cm^2 wooden axle with alternate white and black sides and plastic blades. In motion, the moving axle created a flashing black and white pattern, while the plastic blades generated a noise. The distance that ducklings ran from the stimulus was recorded (Heinz et al., 1979). No significant effects were apparent on ducklings from parents fed methylmercury (0.5 mg mercury/kg diet) for two generations (Heinz, 1976b), but those from parents fed over three generations ran greater distances than controls (Heinz, 1979). Similar results were found with cadmium at 4

mg/kg diet, although 40 mg cadmium/kg diet did not significantly affect the avoidance response (Heinz et al., 1983). No effect of selenium (1-8 mg/kg diet), chromium (20 or 200 mg/kg diet), toxaphene (10 or 50 mg/kg diet) or temephos (1 or 10 mg/kg diet) was found on the avoidance response of ducklings (Heinz & Finley, 1978; Heinz & Haseltine, 1981; Heinz & Gold, 1987; Franson et al., 1983).

Albers & Heinz (1983) found that the mosquito larvicide FLIT-MLO (a petroleum-derived aliphatic hydrocarbon) sprayed onto mallard eggs at the high rate of 140.25 litres/ha significantly reduced the distance run, in response to a fright stimulus, by ducklings hatched from those eggs. However, ducklings hatched from eggs sprayed with normal applications of larvicide or No. 2 fuel oil did not show significant effects.

Methylmercury decreased the responsiveness of ducklings to maternal calls but made them hyper-responsive to danger. In contrast, ducklings whose parents were fed 3 mg/kg DDE were more responsive to maternal calls than controls and less responsive to the frightening stimuli (Heinz, 1976a).

A further test called an 'open field test' measures spontaneous activity. At the Patuxent Wildlife Research Center, the 'open field' is a box 60 cm square by 30 cm high with a 15 W light bulb on the ceiling. Four pairs of photoelectric light sources and sensors divide the 'open field' into nine equal sectors. The spontaneous activity is measured by the number of interruptions in the light beam that are recorded during a test period of approximately 5 minutes (Heinz et al., 1979). Heinz (1979) found no significant effect on the spontaneous activity of ducklings from parents that had been fed methylmercury.

Similarly, Frederick (1976) found no significant effect of lead nitrate concentrations of up to 500 mg/kg on the 'open field' behaviour of mallard ducklings.

Both approach and avoidance behaviours are more directly relevant to survival in the wild than are those tests based on learning ability. However, the results from such tests are somewhat variable and dependent upon the details of the experimental set up.

Changes in nest attentiveness and courtship behaviour can be related to nesting success, but the major disadvantage of such tests is their often extended duration. The complex courtship behaviour of pigeons and doves has been recorded in great detail (Miller & Miller, 1958; Fabricius & Jansson, 1963; Lehrman, 1964), making them ideal subjects on which to study the subtle effects of chemicals. Components of courtship include bowing, driving, billing, nibbling, nest demonstration and nest building. Haegele & Hudson (1973) found that DDE-treated barbary doves (*Streptopelia risoria*) took an average of 2.5 times longer to renest than control birds in undisturbed reproductive cycles. Both the total courtship time and the bowing frequency of barbary doves were reduced during days 59 to 63 of exposure to 10 mg DDE/kg diet and during days 31 to 35 at 50 mg/kg diet (Haegele & Hudson, 1977). McArthur et al. (1983) exposed barbary doves to an organochlorine mixture and found marked changes in the nature and duration of courtship behaviour in a dose-related fashion. Alterations were apparently mediated through the female, with marked asynchrony of the breeding cycle being observed. Keith & Mitchell (1993) reported that 100 mg DDE/kg diet adversely affected the ability of doves to form pair bonds, mate and produce eggs. Tori & Peterle (1983) exposed mourning doves (*Zenaida macroura carolinensis*) to Aroclor 1254 (a polychlorinated biphenyl, PCB) for 42 days prior to pairing. PCB concentrations of 10 and 40 mg/kg diet significantly increased the

length of the courtship phase, whilst the higher dose significantly decreased the behaviour score. There were no significant effects on the length of the pair bond formation period or the nesting phase. However, PCB significantly delayed the onset of nest initiation. Dobson & Westwood (1979) found that organochlorines, such as PCB, dieldrin and DDE, reduced nest building activity of feral pigeons. In fact DDE almost inhibited nest building altogether. Asynchrony in the breeding cycle and inhibition of nest building caused by organochlorines was associated with disruptions of the hormonal feedback system which drives the behavioural responses (Dobson et al., 1977; Dobson & Westwood, 1979; McArthur et al., 1983).

Peakall (1985) states that one of the problems of relating behaviour to nesting success is that it is often difficult to separate the direct embryotoxic effects caused by transmission of the pollutant into the egg from the behavioural effects on incubation and nest attentiveness. Peakall & Peakall (1973) studied the effect of 10 mg PCB/kg diet (Aroclor 1254) on the reproduction of the barbary dove. Embryonic mortality was greatly increased in eggs incubated by parent birds, compared with artificially-incubated eggs. Monitoring the core temperature of eggs suggested that the increased mortality was due to decreased parental attentiveness. Custer & Heinz (1980) found no effect on the nest attentiveness of mallard fed on a diet containing 25 mg Aroclor 1254/kg. White et al. (1983) observed that laughing gulls (*Larus atricilla*) administered with a single oral dose of 6 mg parathion/kg body weight in a field experiment spent significantly less time incubating eggs. However, incubation behaviour had returned to normal within 3 days.

There are many other types of behaviour that have been studied following exposure to pollutants both in the laboratory and in the field. American kestrels (*Falco sparverius*) fed

a diet containing 6 mg DDE/kg diet did not show any significant changes in predatory vigilance or attack behaviour measured as responses to a familiar moving prey model. However, variance in response time increased in control birds, whilst remaining constant in dosed birds (Rudolph et al., 1983). No significant effects on predatory behaviour were observed when kestrels were exposed to acephate (50 mg/kg diet) with or without DDE (35 mg/kg diet) (Rudolph et al., 1984). Similarly, the dominance-subordinate interactions between individuals and the subsequent hierarchical structure of populations is extremely important in the field. Sharma et al. (1976) found that exposure to dieldrin significantly reduced the dominance of dosed mallard drakes compared with controls.

The general activity of several bird species has been monitored and observed by many authors. Both increased and decreased activity could influence the survival of birds in the wild. Doses of 0.24 g dimethoate/m^2 of seeds significantly reduced the mean daily activity (perch hopping) of three granivorous bird species over a 30 day period (Brunet & Cyr, 1992). Hart (1993) reports that starlings exposed to a single oral dose of the organophosphate chlorfenvinphos (3, 6 and 9 mg/kg) showed reductions in flying, singing, preening and feeding, and increased resting periods, although birds recovered within a few hours. Fairbrother et al. (1988) found significant differences in the behaviour of mallard ducklings given a single oral dose of 4 mg/kg methyl parathion. Control birds primarily fed and swam in open water whereas dosed birds predominantly preened, rested or performed other activities. Whitworth et al. (1991) reported that ducklings fed high levels of arsenic (300 mg/kg diet) or boron (1600 mg/kg diet) showed significant increases in resting time and time spent under heat lamps. The time spent in 'alert' behaviours and swimming was significantly decreased. Silver & Nudds (1995) report that adult black ducks (*Anas rubripes*) exposed to 4 mg cadmium/kg diet were significantly more active

than controls. The locomotor activity of bobwhites was significantly decreased by a diet containing 131 mg carbofuran/kg diet; both food intake and body weight were also reduced. No effect was observed at a concentration of 26 mg carbofuran/kg diet or during exposure to carbaryl (1235 mg/kg diet) (Robel et al., 1982). Martin & Forsyth (1993) found no effect on the activity of ducklings released into vegetation sprayed with carbofuran (132 and 264 g a.i./ha).

Peakall (1985) reviewed the behavioural responses of birds to pesticides and other contaminants. A wide variety of behavioural tests was examined, from fairly simple tests such as operant behaviour to more complex tests on breeding and prey capture. It was concluded that, in general, behavioural changes are not a more sensitive indicator of exposure to pollutants than are biochemical changes such as acetylcholinesterase inhibition (see section 3.3).

Behavioural responses are clearly of interest since they may provide a link between the biochemical and ecological consequences of environmental contamination and, therefore, a measure of the effect of a chemical in the field. However, in the absence of standard testing methodology, behavioural observations must be interpreted within the context of each test method. In order to do this, a full understanding of the detailed physiology and behavioural inputs of the system is needed. In other words, the successful use of behavioural endpoints in toxicity testing requires a substantial amount of background knowledge regarding the normal behaviour of the test species. Unfortunately this means that studying the effects of chemicals on behaviour tends to be rather time-consuming and certainly difficult to quantify. The use of behavioural responses in field trials will be discussed in section 4.1.

3.2 Reproductive effects

A number of chemicals have been shown to affect avian reproduction. A wide variety of tests on reproductive performance has therefore been developed and out of these was devised the standard avian reproduction test (see section 2.3). Other tests cover aspects of reproduction, such as egg-laying, development, hatching and growth. Such non-standard tests can offer further insights into the complexities of chemical effects on bird breeding.

The current avian reproduction test is designed to determine the effects of pesticides with chronic exposure patterns on reproductive mechanisms. However, many of the more recent pesticides are much less persistent in the environment and their use patterns are such that the initial contact with these pesticides may come at any time during reproduction. Several studies have been performed for short periods during egg production. Bennett & Bennett (1990) exposed bobwhite quail to methyl parathion (14 to 40 mg/kg) in the diet for 8 days. Body weight, egg production, egg weight and eggshell strength, thickness and weight were all reduced in a dose-related manner, associated with a pesticide-induced reduction in food consumption. Similar findings were reported by Rattner et al. (1982) in a 10 day study with parathion. In 15 day studies with monocrotophos and methamidophos, rates of food consumption and egg production were negatively dose-related during the treatment period, and birds resumed laying after a dose-related recovery interval (Stromborg, 1986a; 1986b). Bennett & Ganio (1991) suggest that the length of a short exposure test could be set for different pesticides as a function of their environmental degradation rate.

Biessmann (1982) exposed Japanese quail to PCB (50, 100 and 150 mg Clophen A60/kg diet) for three weeks during the maturation period (second to fourth weeks of life). In females, PCB caused delayed laying and a diminished laying capacity. However, no PCB effects were detected on ovary and oviduct weight, nuclear volume and lipid content of the thecal gland cells and on plasma 17 β-oestradiol and calcium content. In males, PCB treatment resulted in significant decreases in the amount of seminiferous epithelium and the nuclear volume of Leydig cells. Plasma concentrations of testosterone and 5 α-dihydroxytestosterone were not affected by PCB.

Schafer et al. (1982) administered single oral doses of 71 chemicals at approximately 50% of the LD_{50} value to adult male Japanese quail and monitored fertility (percentage of eggs laid by female mate that were fertile). None of these chemicals proved to be effective enough to warrant further investigation as avian chemosterilants. One of 6 chemicals tested on female quail reduced fertility to 40%.

Bennett et al. (1990) conducted two bobwhite reproduction tests to evaluate how the duration and time of initiation of methyl parathion exposure affected dose-response relationships of reproductive parameters. Pairs of adult birds were exposed for 3 weeks during egg laying in a short-term test and for 25 weeks prior to and during egg laying in a long-term test. Two birds died in the short-term test and 14 in the long-term test, about the same rate when expressed as birds per week. All other dose-related effects observed in the long-term test were also observed in the short-term test. The authors stated that the short-term test had two advantages that reduced variability unrelated to the chemical treatments: the infertile and incompatible pairs could be removed from the experiment, and pre-treatment values could be obtained to serve as controls for each pair.

Longer term studies have been carried out on several bird species with time periods ranging from several months to years. The length of these studies would be too long for standard testing, but the data generated are extremely useful to the overall understanding and improvement of reproductive toxicity testing. Heinz (1979) maintained three generations of mallard ducks on a diet containing 0.5 mg methylmercury/kg. All statistically significant differences between the mercury treated and control birds occurred in the second and third generations. Longcore & Stendell (1977) fed black ducks dietary DDE (10 mg/kg diet) for 2 breeding seasons followed by 2 seasons on untreated feed. Shells of eggs from treated hens were approximately 20% thinner than controls; even after 2 years on an untreated diet eggshells were still 10% thinner than controls. Hens previously exposed to DDE and fed untreated feed for two years still produced significantly fewer surviving ducklings than controls. Mean dieldrin residues in eggs and carcasses were 50 and 150 mg/kg respectively during the treatment period. After 2 years on an untreated diet egg residues were 6 mg dieldrin/kg whilst carcass residues were 12.2 and 3.4 mg/kg for males and females respectively. Control eggs and carcasses contained 0.2 to 0.3 mg dieldrin/kg throughout the experiment. Meyers & Gile (1986) conducted a 2 year reproductive study on mallard using outdoor pond enclosures. No significant reproductive effects were observed for mallards receiving 8 mg/kg chlorpyrifos in the diet. However, birds fed on a diet containing 80 mg/kg diet hatched significantly fewer ducklings per successful nest than controls. None of the ducklings hatched from eggs laid by treated parents survived to 7 days.

Studies on birds can provide valuable information on the interactions between various aspects of breeding, including behaviour, social interaction and hormonal control.

Haegele & Hudson (1973) studied the reproductive performance of 12 pairs of barbary doves fed a diet containing DDE (40 mg/kg diet) over a period of 126 days. Doves exposed to DDE took significantly longer to renest than controls, produced significantly fewer and thinner eggs, and suffered significantly higher mortality of young than controls. Each pair of control birds nested 49 times and raised 35 young during the experiment whereas each pair of DDE-treated birds nested only 33 times and raised 10 young. Tori & Peterle (1983) found significant effects of PCB on mourning dove courtship duration and onset of nest initiation. An organochlorine mixture in the diet of barbary doves had significant effects on the nature and duration of courtship behaviour, on the levels of androgens, oestrogen and progesterone, and on the fledging success of young (McArthur et al., 1983).

It is important in reproductive studies to distinguish between effects on the adults and effects on the eggs themselves. It has already been shown in subsection 3.1 that artificial versus natural incubation can give behavioural insights into the action of chemicals. Therefore, when standard testing methods using artificial incubation show no adverse effects they cannot be taken to demonstrate that no hazard exists because under natural incubation there might also be problems with parental behaviour or egg damage (Heinz et al., 1979). To study the effects of chemicals on the whole reproductive cycle of birds involves natural incubation as an integral part of that cycle (Haegele & Hudson, 1973; McArthur et al., 1983). An organochlorine mixture altered the incubation and brooding behaviour of barbary doves in a dose-related fashion and the mean incubation period was extended by up to three days (McArthur et al., 1983). Several studies have been carried out with captive ducks using parental incubation to simulate more closely the field situation (Finley & Stendell, 1978; Custer & Heinz, 1980; Franson et al., 1983). Some

studies have revealed that chemicals can exert direct effects on incubation which would be missed by the standard reproduction test. Bennett et al. (1991) found that mallard exposed to dietary parathion (400 mg/kg diet) for 8 days showed changes in incubation behaviour ranging from reduced nest attentiveness to complete abandonment. In black ducks fed on 10 mg DDE/kg diet natural incubation increased shell cracking by more than fourfold compared with artificial incubation (Longcore & Samson, 1973).

There are many endpoints which can be used in reproductive studies which are not currently used in the standard reproduction test. Eggshell thickness, as measured in the avian reproduction test, was the first measurement used to determine the effects of environmental contaminants on avian eggshell quality (Ratcliffe, 1967; Hickey & Anderson, 1968). Eggshell thickness has since been monitored in field studies with a variety of species and in the laboratory with standard test species, such as Japanese quail and mallard (Haegele & Tucker, 1974), and with raptors such as American kestrels (Porter & Wiemeyer, 1969; Wiemeyer & Porter, 1970; Lincer, 1975), barn owls (*Tyto alba*) (Mendenhall et al., 1983) and screech owls (*Otus asio*) (McLane & Hall, 1972). A complication with regard to measuring eggshell thickness, especially when trying to interpret data using standard laboratory species, is that of species variation in sensitivity. Chickens and quail are almost completely insensitive, ducks and doves are moderately sensitive whereas many raptorial and fish-eating birds are highly sensitive (Peakall & Lincer, 1996). However, whilst eggshell thickness has proved to be a valuable measure of eggshell quality, it may not necessarily be the most sensitive indicator of shell effects for all chemicals. Carlisle et al. (1986) found that in the mallard shell strength was significantly reduced at a lower dietary DDE concentration than was shell thickness. Bennett et al. (1988) exposed bobwhite to sulphanilamide and compared the relative

sensitivities of breaking strength and shell thickness. Sulphanilamide significantly reduced the shell breaking strength; however, there were no significant differences in shell thickness between eggs from treated birds and those from controls. Scanning electron micrographs of weak shells of normal thickness revealed an abnormal ultrastructure. The measurement of eggshell strength together with thickness would give a much improved indication of the actual effect of chemicals on overall eggshell quality.

Calcium concentrations in the plasma of laying birds could be used as an indicator of adverse effects of chemicals on eggshell quality and egg production. Fairbrother et al. (1990) found that plasma calcium concentrations were about twice as high in egg laying mallards when compared with the rest of the year. Bennett et al. (1990) report that serum calcium concentrations in bobwhites were 2.3 times higher in egg laying females than in males. They exposed bobwhite quail to methyl parathion and observed a dose related decrease in serum calcium concentrations and egg production in laying females. However, food consumption was also reduced by methyl parathion exposure and this would have reduced the overall intake of calcium. It should also be noted that blood calcium levels do not necessarily relate directly to eggshell quality. DDE can cause significant eggshell thinning without affecting blood calcium levels (Peakall et al., 1975). The use of calcium concentrations as an indicator of adverse effects on eggshell quality is not therefore universally reliable.

The effects of chemicals on hormonal changes during reproduction can provide insights into the mechanisms of toxicity. Rattner et al. (1984) reviewed avian endocrine responses to environmental pollutants, concluding that many contaminants have oestrogenic potential and may affect the functioning of the gonadal and thyroidal endocrine

subsystems. Rattner et al. (1982) observed cessation of egg production, inhibition of follicular development and reduced plasma luteinizing hormone (LH) concentrations in bobwhite quail exposed to parathion (100 mg/kg diet) for 10 days. The authors suggested that parathion can impair reproduction by altering gonadotrophin secretion. Similarly, reductions in plasma LH were found in male Japanese quail exposed to 10 mg parathion/kg for 24 hours (Rattner et al., 1986). Dobson et al. (1977) found that PCB caused significant elevations in circulating LH and thyroxine levels immediately before pairing and before oviposition during the reproductive cycle of the feral pigeon. DDE causes a reduction in nest-building behaviour in pigeons; the measurement of LH in the female shows that the lack of stimulus for oviposition normally given by a completed nest delays egg laying (Dobson, 1985). During the post-pairing stage of the barbary dove reproductive cycle an organochlorine mixture significantly reduced plasma androgen levels in males and oestrogen levels in females (McArthur et al., 1983). Progesterone levels in females were significantly reduced during the mid-courtship phase, whilst thyroxine levels were significantly increased during incubation.

Bennett & Ganio (1991) reviewed the methods for evaluating the effects of pesticides on reproduction. They state that, although the standard reproduction test requires information from post-mortem necropsies, this does not specifically include data on the size and weight of internal organs. Treatment-related changes in organ weights can be useful for determining causes of other reproductive effects.

In conclusion, many non-standard tests have shown reproductive effects during short-term exposures. The current test guidelines do not include parental incubation and yet some chemicals have been shown to affect incubation behaviour, and hence hatching success.

There are several other endpoints such as eggshell strength, plasma calcium concentrations and parental organ size and weight which are not included in standard reproductive testing (Bennett & Ganio, 1991). The current standard test methods implicitly assume a simple model for the control of reproduction by environmental variables. The test assumes that light is the principle, if not the only, environmental variable used by birds to time their reproductive cycles. Whilst it is true that birds use light as an indicator of season, and that an inbuilt clock interacts with light cycles to time the development of the reproductive system, it is also true that the onset of breeding uses other environmental and biological cues, such as food, temperature, weather, availability of nest sites, social interaction, etc. (Dobson & Howe, 1990). It would not be possible to include many of these variables within a standard protocol, but it is useful to use such data obtained from non-standard tests in the interpretation of standard test results.

3.3 Biochemical effects

Many assays are now available which allow the researcher to study the effects of chemicals on a variety of biochemical systems. Most fall into two main groups: those based on enzyme inhibition including cholinesterases and carboxyl esterases, and those based on enzyme induction. This particular area is a growing field with the potential to provide predictive information which is potentially less invasive for the bird. It opens up a new area which is particularly relevant to the monitoring of field populations, namely that of biomarkers (see section 4.4). Biochemical assays are now used extensively in the monitoring of organophosphate impacts in the field.

Assays based on measuring the inhibition of cholinesterases have been widely used to assess exposure to organophosphate and carbamate insecticides. The basic procedure is that described by Ellman et al. (1961), with adaptations detailed by Westlake et al. (1980). The assay depends on the release of thiocholine from either acetylthiocholine or butyrylthiocholine; the thiocholine interacts with DTNB (5,5' dithiobis[2-nitrobenzoic acid]), causing a release of 5-thio-2-nitrobenzoate, a yellow anion which absorbs strongly at 412 nm. The change of absorbance is continuously monitored in a spectrophotometer (Walker, 1993).

Bunyan & Taylor (1966) concluded that the most reliable indication of death due to thimet poisoning was the complete inhibition of brain cholinesterase; they found that the inhibition of cholinesterase in the blood and of other esterases in brain and blood was more variable. Ludke (1975) exposed Japanese quail to two cholinesterase inhibitors, namely parathion and carbofuran. They found that brain cholinesterase inhibition exceeding 20% indicated exposure and that inhibition greater than 50% was sufficient for diagnosing cause of death. Rattner (1982) studied factors which might cause variability in the cholinesterase assay and found that underfeeding and exposure to elevated temperatures caused only slight reductions (10-17%) in brain cholinesterase activity in adult male Japanese quail. Cairns et al. (1991) found that a lag time of 2 - 4 hours following exposure to chlorpyrifos was necessary to detect significant brain cholinesterase depression. Thompson et al. (1991) found that the inhibition of serum cholinesterase was a more sensitive indicator of exposure to both triazophos and demeton-S-methyl than brain acetylcholinesterase for up to 24 hours following a single dose to starlings.

Fairbrother et al. (1989) used sequential measurements of plasma cholinesterase activity for monitoring exposure of mallard to methyl parathion (400 mg/kg) during egg laying and incubation. Methyl parathion significantly reduced plasma cholinesterase activity. There were significant differences in plasma cholinesterase activity between untreated birds, but variation between sequential samples from the same bird were 2 and 11 times less than between-bird variation during egg-laying and incubation respectively. A single sample during incubation revealed that plasma cholinesterase activity was more variable than brain cholinesterase activity. Holmes & Boag (1990) exposed zebra finches (*Poephila guttata*) to single oral doses of fenitrothion (1.04, 3.80 and 11.36 mg active ingredient/kg body weight). Maximum brain cholinesterase inhibition ranged from 50% to 75% and for plasma cholinesterase from 78% to 89%. Plasma cholinesterase activity recovered more rapidly than brain cholinesterase.

Driver et al. (1991) studied the relative contribution of the various routes of uptake with regard to brain cholinesterase inhibition by a simulated aerial application of methyl parathion. All four routes of uptake (dermal, preening, inhalation and oral) contributed to the inhibition of brain cholinesterase. Dermal uptake and preening were the major contributors to the inhibition response during the whole 48 hour exposure. Inhalation was the major route of exposure 1 hour after spraying. Oral ingestion resulted in less than 20% inhibition of brain cholinesterase. Martin et al. (1991) found that ducklings led through vegetation (for up to 300 m) sprayed with carbofuran showed inhibition of plasma cholinesterase activity which was inversely related to the spray rate. However, cholinesterase was not affected by the distance walked by the exposed ducklings. Brain and plasma cholinesterase activity did not correlate well.

Hart (1993) attempted to establish relationships between behaviour and the inhibition of brain acetylcholinesterase activity in starlings exposed to chlorfenvinphos. Altered posture was associated with inhibition of cholinesterase to less than 88% of normal levels. Reductions in flying and singing, and increased resting, were associated with levels below 61%. At about 73% inhibition feeding activity and body weight were reduced. The performance of foraging tasks was unaffected by inhibition of acetylcholinesterase to 57% of normal.

Like cholinesterases, carboxyl esterases are also strongly inhibited by organophosphorus insecticides. Avian blood contains a range of carboxyl esterases with a great deal of variation between species. Carboxylesterase activity toward a-naphthyl acetate can be measured by the method of Gomori (1953) and has been adapted by Bunyan et al. (1968) and further modified by Thompson et al. (1991). The naphthol released by hydrolysis of the substrate is assayed by reaction with Fast Red ITR and the absorbance is read at 530 nm.

Thompson et al. (1988a) dosed starlings with organophosphorus insecticides and observed a diurnal variation in serum carboxylesterase activity, but no variation in serum cholinesterase or brain acetylcholinesterase activities. Thompson et al. (1991) found that serum cholinesterase appears to be more sensitive than serum carboxylesterase to inhibition *in vivo* by the organophosphates triazophos and demeton-S-methyl. Fossi et al. (1994b) treated Japanese quail with 10 mg azamethiphos/kg and found that serum butyrylcholinesterase and carboxylesterase activities were inhibited by 88% and 35% respectively.

There are serious limitations to biochemical assays based on enzyme inhibition. They are only relevant to a few classes of compounds, inhibition of esterases in the blood does not give a reliable measure of toxic effect and can only give an indication of rather high levels of exposure to insecticides. The determination of brain cholinesterase inhibition can give a better measure of toxic effect but it is a destructive method and is, therefore, not very suitable for field studies (Walker, 1993).

Recent improvements include the competitive enzyme-linked immunosorbent assay (ELISA) for the quantification of butyrylcholinesterase in avian serum samples (Khattab et al., 1994). The competitive ELISA gives an improved specificity to the assaying of cholinesterase.

Assays based on inhibition have been largely restricted to esterases, reflecting the importance of organophosphorus anticholinesterases. Other biochemical assays include the inhibition of aminolevulinic acid dehydratase (ALAD), used for indicating the presence of biologically significant quantities of lead. Much interest has developed around this assay, mainly because of the potential exposure of waterfowl to lead shot (see section 4.2). The assay has been used with several different species, including mallard (Dieter & Finley, 1978; Eastin et al., 1983), pheasant (Eastin et al., 1983), barbary dove (Scheuhammer & Wilson, 1990) and American kestrel (Hoffman et al., 1985). The precision of the correlation between blood lead and blood aminolevulinic acid dehydratase (ALAD) enzyme activity has been demonstrated by Dieter & Finley (1978), who state that waterfowl exhibiting greater than 50% ALAD enzyme inhibition have been exposed to acutely high lead levels. Developments for other chemicals, such as anticoagulant

rodenticides, would centre around inhibition of the vitamin K cycle and the consequent production of 'undercarboxylated' coagulation proteins (Walker, 1993).

The induction of enzymes responsible for the metabolism of lipophilic xenobiotics can be caused by many different chemicals. The most widely studied involve the hepatic microsomal mono-oxygenases (HMOs) that have cytochrome P-450 as the catalytic centre. HMOs are important in the deactivation and activation of pesticides (Walker, 1983). Methods for monitoring changes in hepatic monoxygenases can either be non-specific or specific. Non-specific methods rely on the fact that induction usually leads to increases in liver weight, microsomal protein per gram of liver and cytochrome P-450 content of liver microsomes. The induction of specific cytochrome P-450 forms can be measured using immunochemical methods (Walker, 1993). Several fungicides, including prochloraz, imazalil and propiconazole, have been shown to be strong inducers of cytochrome P-450 and related monooxygenase activities in Japanese quail (Riviére et al., 1984). Prochloraz has also been shown to induce hepatic monooxygenases in grey partridge, pheasant, chicken and hybrid red-legged partridge (*Alectoris* sp.) (Riviére et al., 1985; Johnston et al., 1989). Johnston et al. (1989) found that the induction of monooxygenases by prochloraz caused a dramatic potentiation of the toxicity of the organophosphorus insecticide malathion. Rattner et al. (1989) reviewed the use of mixed-function oxygenases to monitor contaminant exposure. Case studies include monitoring of seabird embryos, hatchlings, nestlings and fledglings for pollution in the Great Lakes region, Great Island (Newfoundland) and San Francisco Bay, and the monitoring of adult seabirds for PCB exposure from coastal Ireland and Scotland, and lagoons and waste disposal sites in Italy. Other studies include the monitoring of pigeons in India and wintering grebes in Italy for organochlorine exposure. Fossi et al. (1995a) studied the

relationship between feeding habits and interspecies differences in the detoxification ability of the mixed function oxidase system. Omnivorous species, such as gulls, showed the highest activity. Clearly, knowledge of different species-specific detoxification abilities is a useful tool for identifying species potentially at risk in environments polluted with chemicals metabolized by the mixed function oxidase system.

3.4 Direct effects on the egg

This subsection will concentrate on the direct application of chemicals to the egg either by injection, dipping or, more realistically, via spraying. This area of research demands evaluation in its own right because it is impossible in the current reproduction test guidelines to examine exposure of eggs and chicks to chemicals from direct overspray or from parental plumage.

The most widely documented evidence of embryotoxicity following direct exposure comes from studies on petroleum contaminants. Many petroleum crude oils, refined oils, and waste oils are embryotoxic and moderately teratogenic to different species. Albers (1977) found that fuel oil concentrations ranging from 1 to 50 µl applied to mallard eggs caused significant embryonic mortality. Most of the deaths occurred within 72 hours of application. Mallard embryos appear to be most sensitive to petroleum products during the early stages of development (Albers, 1978). Hoffman (1978) revealed that surface applications of microlitre amounts of some crude and fuel oils that coat less than 10% of the egg surface reduced hatching. In contrast, paraffin compounds coating an equal area did not reduce hatching, suggesting that toxicity is not due to asphyxia.

Albers & Szaro (1978) applied 20 µl of no. 2 fuel oil to eider duck (*Somateria mollissima*) eggs at an island breeding colony in Maine, USA. Significant embryonic mortality was observed 7 days later. The results were similar to laboratory studies with artificial incubation indicating that the nest site conditions did not affect embryotoxicity. Albers (1980) exposed adult mallard to oiled water for 2 days during the first week of incubation. The presence of oil on the eggs of exposed ducks was confirmed by chemical analysis. However, no significant effects on egg hatchability or duckling survival were observed. FLIT-MLO and No.2 fuel oil are sprayed on wetlands for mosquito control during spring and summer, but Albers & Heinz (1983) found that normal application rates did not appear to pose a threat to the embryos of breeding birds.

Toxicity of petroleum hydrocarbons appears to be dependant on the polyaromatic hydrocarbon concentration and composition. Hoffman (1979) found that the addition of chrysene to an aromatic mixture of crude and refined oils considerably enhanced embryotoxicity. However, chrysene alone did not account for the toxicity of the oil. The addition of benzo[a]pyrene, chrysene or 7,12-dimethylbenz[a]anthracene to a synthetic petroleum mixture of known composition and relatively low embryotoxicity resulted in embryotoxicity that was enhanced or equal to that of crude oil (Hoffman & Gay, 1981).

The phenoxy herbicides have been widely studied with respect to potential embryotoxicity of spray application because initial studies revealed embryonic abnormalities. Lutz & Lutz-Ostertag (1972) studied the effect of immersing eggs from several laboratory species in concentrated aqueous solutions of 2,4-D and 2,4,5-T. Embryonic malformations were found, including abnormal gonads, stunted growth and fused vertebrae. However,

Kopischke (1972) found no adverse effects on hatchability and no increase in deformities after spraying pheasant and chicken eggs with 2,4-D at a dose equivalent to 0.28 kg/ha. Gyrd-Hansen & Dalgaard-Mikkelson (1974) reported that immersing chicken eggs in 1% phenoxy herbicide solutions had no effect on hatchability; only moderate effects were observed in a 5% solution. Injecting eggs with 2 mg of herbicide did result in a considerable number of malformations. Other studies found no effect on the hatchability of chicken eggs or on the survival and growth of chicks after spraying eggs with 2,4-D (Somers et al., 1974; 1978). More recently, other herbicides have been found to be much more embryotoxic. Hoffman & Albers (1984) calculated the LC_{50}s for aqueous emulsions of paraquat and trifluralin to be equivalent to 1.7 kg/ha and 1.8 kg/ha, whereas the phenoxy herbicides 2,4-D and 2,4,5-T gave LC_{50} values of 215 and 119 kg/ha. Using an oil vehicle the LC_{50} for paraquat was 0.1 kg/ha.

Hoffman & Eastin (1981b) applied three organophosphate pesticides to mallard eggs using formulations and concentrations similar to field applications. The order of embryotoxicity on a kilograms-per-hectare basis was parathion > diazinon > malathion. Parathion had the most pronounced effects, causing stunted growth and many malformations (involving distortion) of the axial skeleton. An LC_{50} for parathion of approximately 2 kg of active ingredient per hectare was calculated. All three pesticides resulted in a significant inhibition of both plasma and brain cholinesterase activity in embryos.

Martin (1990) immersed Japanese quail eggs in aqueous emulsions of carbofuran, chlorpyrifos and deltamethrin at three potential field concentrations and at three stages of incubation. No effects of pesticide exposure on hatchability were detected. Even at twice

the field application, effects were minor and the authors concluded that the three compounds did not constitute a hazard to quail embryos at these exposure concentrations.

Industrial effluents including mineral pigment, scouring effluent, sludge and tannery effluent, and metals (lead nitrate and methyl mercury) applied to mallard eggs at days 3 and 8 of incubation resulted in small but significant reductions in embryonic growth. Oil used for the suppression of road dust was the most toxic of the pollutants tested and only 0.5 µl/egg caused 60% mortality after 18 days of development. Applications of lead nitrate (up to 30 000 mg/kg) did not significantly effect the survival of embryos or increase the number of abnormalities; however, methyl mercury concentrations of 5000 mg/kg significantly decreased survival and increased abnormalities after 3 days (Hoffman & Eastin, 1981a). A significant incidence of malformations occurred at a dose of 1 µg of methyl mercury per egg. The malformations consisted mainly of minor skeletal aberrations and incomplete ossification (Hoffman & Moore, 1979).

Hoffman (1990) reviewed the embryotoxicity and teratogenicity of environmental contaminants to bird eggs. Contaminants in all classes reviewed were shown to cause physiological and biochemical disturbances in embryos or hatchlings, organ damage, or delayed development. The ability of contaminants to pass across the shell and its membranes as well as embryo uptake depend on the compound and its carrier. Pesticides in non-toxic oil were generally more toxic than those in aqueous solution.

Direct application of chemicals to eggs is fraught with difficulties. Injection of eggs is the least environmentally realistic option and is, therefore, difficult to interpret. Immersion, or the dipping, of eggs is also a rather unrealistic route and could cause toxicity through

suffocation. The most realistic exposure route is via spraying, although this also raises questions regarding actual exposure. The choice of laboratory species may be critical because eggshell porosity and therefore interspecies susceptibility may vary. Mallard eggshells are more porous than quail eggshells and are seen as more suitable for testing. Passerine eggshells are thinner and may be even more susceptible (OECD, 1996). However, eggs of ground-nesting species, such as waterfowl and galliformes (groups which include the common test species), are more likely to be exposed directly to pesticides and other chemicals in the field.

4. Field studies

4.1 Field trials

Field testing is an essential part of the safety evaluation of some chemical products. Field trials can form either a regular part of the evaluation process or a conditional requirement dependent on the level of risk predicted from other information. Trials can resolve doubts about a chemical's safety, but only if they are well designed, carried out by experienced staff on carefully chosen sites and are interpreted by experts. However, good field trials are difficult to design, time consuming and expensive (Riley, 1990). Greig-Smith (1990) identified two contrasting types of field trial design: intensive trials which usually involve detailed study of a few sites, or extensive trials with many sites examined in less detail. Fite et al. (1988) produced a guidance document on terrestrial field studies performed under the data requirements of the United States Federal Insecticide, Fungicide and Rodenticide Act. They state that the purpose of such field studies is either to refute the assumption that risks are real or to provide some quantification of the risks. Because answering such questions can be expensive, field studies should ideally provide the necessary data in a practical and economic manner. Fite et al. (1988) divided field studies into two categories: screening and definitive. Screening studies attempt to find whether the hazard suggested by lower tier laboratory or pen studies exists under actual usage. Such studies are usually limited to the observation of acute toxic effects such as direct poisoning and death, but sometimes also check for sublethal effects on behaviour or longer term survival. Methods include carcass searching, radiotelemetry, studies of cholinesterase inhibition, residue analysis of carcasses and food items, and behavioural observations, together with density and diversity estimates. It is important that the area chosen is representative of the areas where a particular pesticide will be used and that

study sites are randomly selected within the geographical area. The authors propose that, to maximize the hazard presented by a pesticide, the selected study sites should contain species which would be at highest risk from an application. A more detailed investigation (definitive study) can be undertaken to quantify the magnitude of the impacts identified in the screening study or from other information. Whilst a screening study can monitor the proportion of the local population that is expected to be exposed, the definitive study examines a sample of the entire local population to quantify the magnitude of acute mortality, reproductive and survival impairment. It is important to understand the autecology of the species being studied, the applicability of the methods used to the pesticide use pattern, and the study site characteristics. Many of the methods used in screening studies are applicable in definitive studies, including mark and recapture techniques, territory mapping methods, nest monitoring and analysis of the age structure of populations.

Carcass searching is the most direct way to determine whether mortality has occurred. The main problems are that most carcasses are not found, and there is no way of knowing how many birds have died in total (including some outside the study area) or of determining the number of individuals at risk. Recovery of carcasses can be very low; Balcomb (1986) found that 60% to 90% of carcasses placed in the field disappeared overnight. Fischer (1990) found that calculations of percent mortality using different formulae can give very different estimates. For scarce species, detection of a single death could result in greatly exaggerated estimates. It is suggested that, although species-specific data are desirable, percent mortality estimates may be more meaningful if based on groups of species which are likely to have a similar exposure to the test compound because of similar feeding behaviour; however, this makes no allowance for species

differences in sensitivity. Mineau & Collins (1988) presented a simple model which defines the likelihood of success of the carcass searching technique as a function of a number of search parameters or fixed environmental attributes. The probability of detecting an effect is calculated as the probability of recovering dead birds and having an assay to identify a proportion of carcasses as treatment-related. The model assumes treatment on day 0 and requires the following variables: local population size, background and pesticide-induced mortality rates, carcass removal rates through scavenging, the type of search pattern, the efficiency of finding carcasses, the assay sensitivity and assay specificity. The authors conclude that carcass searching has a role to play in field trials but that results have to be treated with caution. The elements of searching efficiency and carcass removal by predators can be estimated by field trials, but the lack of knowledge regarding the behaviour and fate of birds exposed to a pesticide must be considered when evaluating the accuracy of these techniques (Dingledine & Jaber, 1990).

Although in field tests, emphasis is usually placed on searches for carcasses, direct observation of bird activity before and after applications can also provide useful information: for example, on whether the test site is representative of typical usage conditions, the likely exposure of species to the pesticide, and the effects of the application on the numbers and activity of local birds (Fletcher & Greig-Smith, 1988). Observations of bird behaviour can be effective indicators of the effects of insecticides (Richmond et al., 1979). Bunyan et al. (1981) carried out observations of bird activity during an intensive field trial, and ranked the birds according to a risk index. The risk index was based on the assumption that exposure of each species to the pesticide was a function of the total numbers seen during sixty observation periods and the number of observation periods during which they were observed. However, the census method had shortcomings: for

example, it ignored the behaviour of birds observed, so that both resting and feeding birds contributed equally. It also failed to include certain species, such as the woodpigeon (*Columba palumbus*), which was commonly observed on the site but not during the observation periods. Nevertheless, the method appeared to highlight differences between species with regard to risk which would not have been picked up during carcass searching. Hart (1990) concluded that behavioural observations have a critical role in field tests, especially where no adequate measures of mortality or reproductive effects are available. However, the variability of the observed behaviours can be such that any meaningful changes are difficult to detect (Hart et al., 1992; Thompson et al., 1992). To have any chance of detecting effects, observations must be both systematic and quantitative, and focused on behaviours which are closely related to survival or reproduction. There should always be collection of control data both before treatment and from untreated areas, with repeated observations of marked individuals. It is important that biases are avoided including observer bias, behaviour as a determinant of exposure, and the effect of movement and behavioural changes (Hart, 1990).

Capture-recapture methods have been used successfully for studying small mammals in farmland. However, the method has been underused for sedentary bird populations. There are several methods available, including colour marking, radio-tagging and ringing. Observation of birds which have been colour marked has been found to be extremely time-consuming. Radio-tagging has so far been restricted to single large-bodied indicator species, thus limiting its suitability as a method for field trials. It has been used, for example to study the impact of rodenticides on owls (Hegdal & Blaskiewiz, 1984; Hegdal & Colvin, 1988; Merson & Byers, 1984). Further miniaturisation and automation may allow application of this technique to smaller species. Even so there are difficulties with

the technique, such as the effects of capture and the equipment itself, and the fact that movement may mean that individuals are outside the treated areas at the time of application (Dingledine & Jaber, 1990). Bird ringing as a marking method is by far the best although it is still subject to the movement problem. Pre-treatment and control recapture data will identify which species or what proportion of the population is sedentary and allow population declines in the treated area to be identified (Edwards, 1990). Studies in the breeding season, when birds are singing and territorial, give most opportunity for detecting declines in numbers, and the species most affected. Statistical models that have been commonly applied to mark recapture data usually assume that capture-recapture probabilities are constant for all birds. Madrigal et al. (1993) outlined an alternative statistical approach based on the beta-binomial distribution that allows for heterogeneous capture-recapture and gives more precise estimates of population size under conditions of heterogeneous probabilities.

Many biochemical responses can be monitored during field trials. However, only inhibition of esterases has been used to any extent, a biomarker which indicates both exposure and effect for organophosphates and carbamates (Bunyan et al., 1981; Busby et al., 1987; DeWeese et al., 1983; Hart et al., 1992; Niethammer & Baskett, 1983; Zinkl et al., 1984; Hardy et al., 1993) (see subsection 3.4). Rapid progress in molecular biology, and the ever-improving sensitivity and specificity of assays, means that biochemical methods are becoming more and more integral to field studies (Walker, 1990a). Other enzymes that have been monitored during field trials include carboxylesterase and glutamate oxaloacetate transaminase (GOT) (a good indicator of cellular damage) (Tarrant et al., 1992; Thompson et al., 1988b; 1992). The use of biomarkers in field trials is in its infancy, but development may be rapid in the near future.

Nesting studies can provide a sensitive measure of pesticide effects, but ideally require a large sample of nests and replication of treatments (Richmond et al., 1979). Nests should be checked regularly to provide adequate coverage of all phases of the nesting cycle. Bairlein (1990) found that detailed nest monitoring, coupled with the analysis of reproductive parameters in experimental populations of hole-nesting passerine birds, was a reliable method for field testing pesticides. However, such a method would be limited to only a few study plots and species. A major limitation of nesting studies is the need for adequate control samples. Comparable control plots are necessary to measure pesticide related differences in the parameters being monitored. Finding pesticide-free control areas with similar numbers of nests and species within similar habitats can be difficult (Dingledine & Jaber, 1990). Several studies have shown that monitoring nests can reveal sublethal effects of pesticides in the field. Starlings (*Sturnus vulgaris*) nesting in boxes in an area treated with methyl parathion at an application rate within the recommended range (1.4 kg a.i./ha) showed no significant reduction in hatchability of eggs or in the mean number of young fledged per nest; however, the total number of nestlings fledging from the treated field were significantly lower than from the control field (Robinson et al., 1988). Rondeau & Desgranges (1995) studied the effects of insecticide use (diazinon, dimethoate and insecticidal soap) on breeding birds in Christmas tree plantations. The authors found significant species differences in sensitivity based on mean hatching rate and mean survival rate of chicks. For example, American robin (*Turdus migratorius*) eggs were sensitive to dimethoate and diazinon, particularly when spraying was carried out early in the incubation stage. In the song sparrow (*Melospizsa melodia*) nestlings were sensitive, either after direct spraying with diazinon or when dimethoate applications had been made at the egg stage. Care must be taken when interpreting results from nest

studies to distinguish between direct toxic effects of pesticide application and indirect effects such as reduced food supply. Pascual & Peris (1992) carried out a field study to assess the effects of two aerial sprayings of a dust formulation of cypermethrin (15 kg/ha) on the food supply and breeding success of the blue tit. Significant effects on nestling mortality, daily survival rate, nest success and nestling weight were observed. However, the authors suggest that the effects of spraying were most likely to be due to shortage of food rather than direct toxicity. It should be noted that direct toxicity could not be ruled out altogether because routes of exposure were not assessed, carcasses of dead nestlings were not analysed and the behaviour of the adults was not studied. Powell (1984) found that a single application of the insecticide fenthion (52 g/ha) resulted in significant reductions in the abundance of the principal food item (noctuid larvae) of red-winged blackbird nestlings, although this did not result in significant adverse effects on any of the reproductive parameters measured.

Edwards et al. (1979) found that bird territory mapping was a reliable method for studying changes in bird populations following the application of a pesticide. Removal experiments were used to test whether the census method would reveal the number of birds removed and assess the rate of reinvasion. Satisfactory censuses were achieved for all four common bird species studied. In each case the percentage of the population removed was revealed correctly by the 48 hour post-removal census.

Avian surveys can provide information on abundance, species richness and species diversity which can be used in evaluating the potential for avian exposure to the test compound. Surveys can also help to identify species most likely at risk. However, other factors such as weather, habitat quality and edge habitat effects, especially in arable

farmland, can potentially effect avian survey results by increasing variability and reducing the possibility of detecting treatment-related changes in bird numbers (Dingledine & Jaber, 1990). Using the grey partridge as an example, Aebisher & Potts (1990) showed that for a field study to detect actual effects of pesticide application, it is important to be aware of the minimum sample size necessary. They found that for an average density of partridges (5 pairs/km^2), a study area of at least 25 km^2 would be required to measure changes in numbers of birds from year to year. The impact of pesticide-induced insect mortality upon chick survival was unlikely to be detected, unless insect mortality exceeded 50%, and detecting the impact of insect mortality on chick survival required a minimum study area of 7 km^2. Field trials of pesticides and associated measurements of bird population densities, if they are to be meaningful, must be carried out at appropriate times of year. It is also necessary for trials to be conducted when the pesticide would normally be applied. This is because many insectivorous birds are migrants, physiological condition varies through the year, the diet of nestlings can often be limited and nesting is confined to periods when food is reasonably plentiful (Evans, 1990).

The drawbacks of each approach to field studies were discussed in a workshop held in Cambridge in 1988 to attempt the standardization of procedures for the field testing of pesticides. It was concluded that there is uncertainty in many aspects of field assessments of pesticide safety and that both flexibility and expert knowledge are essential during the planning, execution and interpretation of field trials. Field testing is an essential part of the safety evaluation for some chemicals and the need for such trials should be based on a variety of factors rather than a fixed automatic trigger. Field trials should represent typical conditions of use. Methods can be qualitative or quantitative, but must be sufficiently sensitive to detect any adverse effects that occur. The workshop concluded that the

accuracy of extrapolation from effects on field trial sites to estimating the impact on wildlife populations remains uncertain (Greig-Smith et al., 1990).

It is clear that the different methods and techniques used in field trials have both strengths and weaknesses. It is important to be aware of the possibility of sample bias. An appreciation of the variability inherent within natural systems is vital for the understanding and interpretation of field study results. Although statistical evaluation of results is important, it is an understanding of what is biologically meaningful which makes the trial successful. To interpret field trial data requires a biological interpretation based on sound scientific judgement and an understanding of the ability or limitations of methods to measure changes in the wildlife community (Dingledine & Jaber, 1990). Each field study is unique although some elements may be common among many studies. The key to understanding and interpreting field studies is a sound protocol (Fite et al., 1988). Within broad guidelines, each field study must be customized to the particular pesticide under evaluation. The knowledge of the probable environmental behaviour and toxicological profile of the pesticide, and the detailed biology of various wildlife species in those habitats, will determine the specific methodology and sampling techniques to be used in the field assessment (Hardy, 1990). Cooke (1990) points out that a level of acceptability needs to be established for any given field trial with regard to damage and/or mortality. In the absence of good quantitative data, a value judgement of the acceptability has to be made and should include the consideration of previous case histories if possible.

In conclusion, field trials usually take place between laboratory testing and final registration of a particular chemical. They can involve determining patterns of exposure (eg. whether organisms consume pesticide granules or baits), assessing repellency (by

observing the utilization of a treated field by birds or other animals), and measuring effects such as the survival of birds, residue analysis of dead or live-caught birds, biochemical studies and other aspects. Trials should be an extremely important check on any findings made during testing, can reveal previously unsuspected effects and aid in the difficult extrapolation from laboratory to field. However, to be able to interpret the results requires a well structured design, a well defined aim, in-depth knowledge of study sites and expert judgement. It is, as yet, unclear whether any of the current designs will adequately meet all of the criteria needed to assess field effects of pesticides though each has some features of interest.

4.2 Monitoring

The purpose of monitoring may range from reassurance that predictions are correct to safeguarding against unforeseen hazards or failures of control (Moriarty, 1988). For a monitoring programme to be useful, the objectives need to be determined from the start because they dictate the type of sampling that is required in order to obtain reliable conclusions. Moriarty (1988) lists three types of information that can be gained from a well designed monitoring programme:

i) rates of release of pollutants into the environment

ii) degree and changes of environmental contamination

iii) biological effects.

In the UK, a comprehensive scheme by the Ministry of Agriculture, Fisheries and Food for investigation of wildlife poisoning from the use of approved pesticides has been in operation for many years. The scheme involves the investigation of reported bird and

mammal mortalities on farmland from a combination of field visits, *post mortem* examinations, residue analysis and assays for esterase activity. The results from the scheme are published on an annual basis (eg. Fletcher et al., 1995; Hardy et al., 1986). Several major problems have been identified by the scheme that had not been predicted prior to registration. The monitoring programme has also revealed deliberate abuse and careless misuse of pesticides (Greig-Smith, 1988). The importance of such a post-registration scheme is that it covers several aspects which are not properly addressed in experimental trials, and can reveal effects on a wider range of species. The scheme assigns incidents to one of five categories: approved use, misuse (careless, accidental or wilful failure to adhere to the correct practice), abuse (deliberate, illegal attempts to poison animals), unspecified use and veterinary use. Deviations from recommended practice can thereby be detected and this may lead to follow-up experimental studies. The problems of disturbance in intensive field trials and effects on rare species which could not be subjected to experimental risks can be monitored. Monitoring provides a constant check on effects occurring in a broad range of habitat and weather conditions that could never be fully simulated in trials (Greig-Smith, 1990). However, incidents are defined as such only when residues of pesticide (or associated biochemical changes) are definitely identified. This will consistently underestimate effects of pesticides which are rapidly metabolized without leaving measured traces.

Many incidents involving birds have been reported in conjunction with the use of pesticides and, in particular, the acutely toxic organophosphorus and carbamate insecticides (Smith, 1987). Most reported incidents usually take place over a relatively short time-period and occur in a defined geographic area. Several poisoning incidents involving geese were reported after the spraying of turf, particularly at golf courses, with

diazinon (Zinkl et al., 1978; Frank et al., 1991). Many bird kills have resulted from both misuse and normal application of granular forms of the carbamate carbofuran (Flickinger et al., 1986). Balcomb (1983) reported secondary poisoning of red-shouldered hawks with carbofuran and a recent investigation in Switzerland also revealed secondary poisoning of raptors via contaminated earthworms (Dietrich et al., 1995). Since 1980, the Swiss Ornithological Institute has registered the cases in which dead or intoxicated raptors were reported in conjunction with assumed or observed pesticide application (Jenni-Eiermann et al., 1995 cited in Dietrich et al., 1995). Between 1980 and the early 1990s a total of 90 dead or severely ill buzzards (*Buteo buteo*), black kites (*Milvus migrans*) and red kites (*Milvus milvus*) have been registered, usually during the spring. Intoxication by carbofuran appeared highly likely as indicated by the use of the pesticide in the areas where the carcasses were found. The crops of eight buzzards were analysed and found to contain carbofuran residues (Dietrich et al., 1995).

The Institute of Terrestrial Ecology in the UK has also carried out a monitoring programme for wildlife incidents since 1969 (Osborn, 1985). These incident investigations have included the Irish seabird wreck in 1969 (NERC, 1971), alkyl lead poisoning in the Mersey in 1979 (Bull et al., 1983), lead poisoning in swans from 1975 (French, 1990) and the North Sea bird wreck in 1983 (Osborn et al., 1984), and more recently oil spills off the Shetland Isles and Pembrokeshire coast (Malcolm et al., 1996).

In a monitoring scheme designed to indicate chemical release, change in contamination levels and effects, the Institute of Terrestrial Ecology at Monks Wood (UK) has been monitoring tissues and eggs from a variety of predatory and fish-eating birds for over 30 years. The programme was started in 1962 when there were serious concerns over the

effects of organochlorine insecticides and organomercury fungicides on several bird and mammal species. The scheme uses carcasses sent in by the public which are assessed for effects by *post mortem* and exposure by chemical analysis. The early monitoring work helped to demonstrate the adverse effects of the organochlorines, and eventually contributed to a ban on their use in the UK and elsewhere (Cooke et al., 1982; Newton et al., 1995). The results of an analysis of this long-term sampling programme (Cooke et al., 1982) confirmed many of the early theories regarding the accumulation of organochlorine insecticides. Birds with the highest residues of DDT or its metabolites were either terrestrial predators feeding on other birds or aquatic predators feeding on fish. Thus, residues of DDE in the liver of the peregrine falcon (*Falco peregrinus*), with birds as its principal dietary component, averaged 7.56 mg/kg, whereas for the kestrel (*Falco tinnunculus*) feeding primarily on mammals, mean DDE levels were 1.10 mg/kg over a period from the early 1960s to the mid 1970s. Cooke et al. (1982) found marked geographical differences throughout the UK, related to usage patterns of DDT, and also marked seasonal changes in residues. These seasonal changes in residues appeared to relate more to seasonal changes in body composition than to environmental availability of pollutants. Currently the monitoring programme includes analysis of livers for organochlorine and mercury from predatory and fish-eating birds (Newton et al., 1993), the analysis of eggs (Newton et al., 1989; Newton & Galbraith, 1991; Newton et al., 1990a) and the monitoring of barn owl (*Tyto alba*) carcasses for rodenticides (Newton et al., 1990b).

In contrast to analysis of bodies found dead, other programmes deliberately and systematically sample either whole birds, eggs or tissue samples from the field to establish more evenly sampled populations. Colonial nesting water birds in the Great Lakes region

have been intensively monitored for over 30 years with eggs and tissues being systematically analysed for organochlorines. Since the late 1960s, many reproductive impairments, including chick deformities, embryo mortality and low hatching success have been reported in terns and gulls (Gilbertson, 1974; Gilbertson et al., 1976; Gilbertson & Fox, 1977; Hoffman et al., 1987; Kubiak et al., 1989). However, although tissue and egg samples contained increased organochlorine levels, no cause-effect relationship between specific contaminants and reproductive failure could be established. Similarly, severe reproductive effects such as embryonic mortality and abnormalities have been reported in irrigation ponds in California. These effects have been linked with the impact of selenium on a variety of aquatic birds (coot, mallard, grebes and waders) (Ohlendorf et al., 1986; Hoffman et al., 1988).

Seabirds have been used extensively as indicators of marine pollution (Gilbertson et al., 1987). The Canadian seabird monitoring project has examined seabird eggs since the late 1960s and a range of species from the Atlantic, Arctic and Pacific Oceans has been studied. Elliott et al. (1989) sampled eggs from the Pacific coast between 1971 and 1986, and Pearce et al. (1989) analysed seabird eggs from the Northwest Atlantic between 1968 and 1984. Eggs from Arctic species were collected from Prince Leopold Island (Peakall & Nettleship, 1987), whilst Elliott et al. (1988) analysed eggs of northern gannets nesting on Bonaventure Island (Eastern Canada) between 1968 and 1984. The results show that, since the early 1970s, the mean residues of most organochlorine pesticides and industrial compounds have declined in the food chains of inshore and continental shelf seabirds on both coasts of Canada (Elliott et al., 1992). Similarly, Moksnes & Norheim (1986) analysed herring gull (*Larus argentatus*) eggs collected from the Norwegian coast between 1979 and 1981, and found a significant decrease in DDE levels compared with eggs

collected in 1969, while PCB levels had not declined significantly. Newton et al. (1990a) analysed gannet eggs for DDE, HEOD, PCBs and mercury from several colonies around the British coast during the period 1971 to 1987. Generally levels of organochlorines declined during the period from the early 1970s to 1983; however, increases were detected between 1983 and 1987. Mercury levels were very variable with increases during the study period at four colonies and decreases at two colonies.

The use of feathers as indicators of mercury pollution was investigated extensively in Sweden during the 1950s and 1960s as a result of the toxic effects of alkylmercury seed dressings on both seed-eating and predatory bird species. The general mercury level of various bird species can be estimated from the mercury content of their feathers which is related to the amount of mercury circulating in their blood during feather formation (Berg et al., 1966). Jensen et al. (1972) analysed goshawk (*Accipiter gentilis*) feathers which had been collected between 1815 and 1965. They found a dramatic increase in mercury levels between 1940 and 1965 associated with the introduction of alkyl-mercury type seed dressings. A ban on alkyl mercury dressings in Sweden in 1966 led to a dramatic decrease in the mercury content of goshawk feathers, an example of monitoring used to study the effectiveness of control. Since the late 1960s the use of bird feathers as a non-invasive method of monitoring pollutants has gained wide usage. However, pollutant concentrations in the feathers of bird species are affected by the age of the bird, the time of year it was caught, the moult pattern and, in particular, the exposure at the time of feather formation (MARC, 1985; Pilastro et al., 1993), so these factors must be taken into account in any interpretation of findings. Goede & De Bruin (1986) state that the feather can be used as an indicator for the presence or absence of mercury, zinc, lead and selenium; however, for zinc the feather vanes can be used only after completion of feather growth,

and for selenium and lead the time elapsed since feather formation must be known in order to interpret the data because contamination can be deposited by secretion from the preen gland. Furness et al. (1986) found that the amount of mercury stored in body tissues had more influence on levels in feathers than did the amount of mercury in the diet. They found that body feathers provided the most representative sample for estimating whole-bird mercury content. Lead and mercury levels have also been found to be higher in the distal fully formed portion of the growing feather compared with the proximal, growing portion which has a residual blood supply; however, no such differences were found for cadmium and selenium (Burger & Gochfeld, 1992). Migratory birds can show extremely large variations, because their mercury contents have been derived from more than one area. However, for mercury at least, it would appear that the use of feathers from museum specimens, when analysed statistically, gives an acceptable method of determining past environmental contamination. Bird collections in museums would appear to represent a large source of historical data as long as the results are interpreted with care (MARC, 1985).

Eggshell collections were used to detect and date the effect of DDE on avian eggshell thickness (Anderson & Hickey, 1972; Anderson & Hickey, 1974; Hickey & Anderson, 1968; Ratcliffe, 1967; Ratcliffe, 1970; Newton, 1986). Museum specimens have also been successfully used to investigate both the structural and functional alterations induced by DDE in eggshells. Since these early studies, many researchers have found evidence of eggshell thinning in raptor and other species. One of the most detailed studies to show eggshell thinning, after the introduction of DDT and subsequently the recovery after strict control of this insecticide, involved the sparrowhawk (*Accipiter nisus*) (Newton & Haas, 1984). Similar effects have also been reported for several seabird species. Elliott et al.

(1988) found that the eggshell thickness of gannets (*Sula bassanus*) from Eastern Canada had decreased by 17% in eggs collected in 1969 when compared with pre-1947 eggs. Eggs collected in 1984 showed a 20% increase in eggshell thickness over eggs from the late 1960s. Burger et al. (1995) also report an increase in seabird eggshell thickness between the 1970s and the 1990s; for some species eggshell thickness increased by nearly 50%. The role of DDE as the main causal agent has also been confirmed by experiment (see section 3.2).

Harrison et al. (1990) developed a non-invasive technique for monitoring the exposure of barn owls to rodenticides. They found that caged owls fed mice containing residues of flocoumafen excrete a consistent percentage of these residues in regurgitated pellets, thus collecting and analysing pellets would provide a good indication of rodenticide exposure in the field. The technique has been successfully used in the field to monitor the exposure of owls to 'second-generation' rodenticides (Gray et al., 1994).

Lead poisoning in waterfowl, and swans in particular, is a good example of how monitoring can highlight a particular problem and suggest ways of changing the situation. Here monitoring was used to assess population size, degree of contamination of individuals, geographical distribution of the pollutant and repopulation. Lead poisoning through ingestion of gunshot and fishing weights is now a well-known phenomenon. Waterfowl ingest shot along with, or mistakenly for, grit which is used in the breakdown of food in the gizzard. Within the gizzard, shot is eroded and dissolved and toxic lead salts are absorbed into the bloodstream, causing death by paralysis. Lead poisoning in mute swans (*Cygnus olor*), following ingestion of lead fishing weights, was first recognized in 1973 (Simpson et al., 1979). Since that date, *post-mortem* and blood lead

examinations have shown lead poisoning to be a major mortality factor in mute swans (Birkhead, 1982; Sears, 1988). A report by the Nature Conservancy Council's Working Group in the United Kingdom discussed the problem of swan deaths attributable to lead poisoning (NCC, 1981). The report revealed that mute swan populations in the U.K. showed 8% to 15% decreases between 1955 and 1978, although there were large geographical differences. In the years 1980 and 1981 the Ministry of Agriculture, Fisheries and Food conducted *post mortems* on 288 mute swans. They reported that 39.2% of the swans had died of lead poisoning; however, again there were large geographical differences, with 50% of the English birds dying of lead poisoning but none of the Scottish birds. *Post mortems* on 299 mute swans carried out between 1973 and 1980 revealed gun-shot in only 5 birds. It was found that other swan species (Whooper *Cygnus cygnus* and Bewick *Cygnus columbianus*) were more likely to contain gun shot because of the differences in feeding habitat; two-thirds of whooper and Bewick swans dying of lead poisoning on the Ouse Washes contained gun shot. The report acknowledged that the problem of lead use by anglers has not changed appreciably in 150 years, yet the elevated swan death rate is a recent phenomenon. The report suggests that the most likely explanation is a change in the distribution of aquatic plant life. In recent years, marginal and submerged plants have been killed by pollution from boats, and more significantly, by the use of herbicides to keep channels clear. The lack of marginal plant life would make lead shot more accessible and, therefore, more available to swans. These changes in the vegetation and the increased incidence of discarded lead weights has led to an increasing number of lead poisoning incidents. A subsequent ban on the use of lead fishing weights, and the use of alternative materials, has generally resulted in an improvement in the situation. The number of swans dying of lead poisoning has declined and blood lead values have similarly fallen (Sears, 1988).

Gun-shot is a more important source of lead in birds in North America. Trainer & Hunt (1965) estimated that 1700 Canada geese (*Branta canadensis*) succumbed to lead poisoning in Wisconsin between 1940 and 1965. Bagley et al. (1967) collected dead and dying Canada geese and found shot in the gizzard and elevated lead levels in liver, tibia and kidney. Anderson (1975) examined 1500 waterfowl at Rice Lake, Illinois, USA. The majority of birds were lesser scaup (*Aythya affinis*); 75% of individuals of this species contained at least one lead pellet in the gizzard. Lead levels averaged 46 mg/kg in the liver, 66 mg/kg in kidney and 40 mg/kg in wing bone. The incident occurred following a period of drought which killed food plants, and with a return to normal water levels, plants began to grow again but lead pellets were still more readily available in the feeding sites.

For effects of lead on wildfowl, monitoring showed that contaminated areas would continue to cause mortality in birds recolonizing from clean sites. It also related site management to poisoning (the distribution of the lead was wider than the effects) since vegetation type determined lead availability. Here monitoring of pollutant distribution and effect was profitably combined with information on the environment itself.

The long-term monitoring of bird population size and success is a huge subject area in itself and cannot be covered in detail here. However, it is important to highlight the usefulness of such data in the context of pollutants. Knowledge of population trends is vital for an understanding of the effects of chemicals on birds in the field. As mentioned above, DDT and its metabolites, principally DDE, have been implicated in reproductive effects on birds in the field. Large population declines in some bird species, mainly birds of prey, have been blamed on DDT or on combinations of DDT and other persistent

organochlorines. The populations of many species of birds of prey were monitored throughout a period of high DDT use. This was done by large scale surveys and studies of population dynamics (Henny, 1977; Lindberg, 1977; Ratcliffe, 1972; White & Cade, 1977; Newton, 1986), migration counts at observation points (Edelstam, 1972; Hackman & Henny, 1971; Nagy, 1977; Rosen, 1966; Ulfstrand et al., 1974), and by sample counts in particular areas (Ash, 1965; Bezzel, 1969). The subsequent recovery of populations was correlated with the reduced use of persistent organochlorine insecticides (Newton, 1979; 1984). To be able to draw conclusions requires long-term monitoring of bird populations alongside the monitoring of chemicals. However, such programmes are expensive and it is essential that the aims are clear from the outset.

Two good examples of long-term monitoring programmes are those on the sparrowhawk (*Accipiter nisus*) and the grey partridge. The sparrowhawk has been extensively studied in southern Scotland for more than 25 years (Newton, 1988; Newton & Bogan, 1978; Newton et al., 1981; Newton et al., 1979). During this period a detailed picture of the ecology of this raptor has been built up and this has been coupled with the monitoring of organochlorine pesticides in eggs and carcasses. Data revealed that population declines could be attributed both to the death of adults by dieldrin and to reduced reproductive success through eggshell thinning caused by DDE. Studies were also able to monitor the population recovery of the sparrowhawk following reductions in the use of these organochlorine insecticides. A long-term study of the grey partridge has been carried out by the Game Conservancy in the South of England (Potts, 1986). During this study a large amount of data has been gathered regarding the ecology of the grey partridge. It has been possibly to study the influences of a variety of factors on the population decline of this farmland species. Factors such as nest predation, starvation amongst chicks, shortage of

nesting cover and shooting rates have been incoporated into a computer model. Simulations of the partridge population can be studied using the model which also help to identify the actual factors which have contributed to the decline. It was found that population declines were due to high chick mortality accentuated by increased egg predation. The high chick mortality was primarily caused by the use of herbicides which indirectly eliminated insects by removing their food source (weeds). It is only with such detailed, long-term studies that predictions at the population level can be confidently formulated. Newton (1988), using the long-term monitoring data set on the sparrowhawk, was able to predict critical pollutant levels that were associated with population decline.

The major problem with monitoring programmes is to link the chemical analysis with observed effects in the field. For example, eggshell thickness was correlated not only with DDE but with other organochlorines which were themselves correlated with DDE. It was therefore difficult to determine solely from the field data exactly which chemical was responsible for the effect. This problem was addressed by Newton & Bogan (1978) using non-experimental methods. They conducted a statistical analysis of their data that showed a correlation between DDE and shell thickness, egg breakage, egg addling, and hatching failure, in addition to a correlation between DDE, PCB, and dieldrin residues. After multivariate analysis, DDE alone appeared to be responsible for eggshell thinning and egg breakage. Many other monitoring programmes have revealed correlations between egg DDE residues and the degree of eggshell thinning in raptors (Newton, 1979). However, experimental studies are required to establish an actual cause and effect relationship. In the case of DDE, experimental studies did indeed confirm the correlations obtained from field studies.

Monitoring can focus on a particular area, chemical or species. Programmes can include chemical analysis of tissues from carcasses, analysis of feathers and blood samples from live birds, ecological studies of particular species and population censuses. Long-term monitoring can reveal trends of use and misuse of chemicals, as well as effects that are not apparent in short-term trials. However, there are many problems associated with monitoring and care must be exercised in drawing conclusions. Correlation does not necessarily mean causation and, therefore, many of the findings from monitoring studies need to be confirmed by experimentation.

4.3 Bioaccumulation

Much of the discussion surrounding predictive testing is centred around immediate effects; but much of the long-term risk from chemicals, and especially some older pesticides, stems from their tendency to bioaccumulate within animals. Any assessment must therefore address this aspect for a more complete prediction of chemical effects.

The more persistent a pollutant is in the environment, the more relevant becomes the question, by how much does position in the food chain determine or influence the mass, or body burden, of that pollutant in individual animals (Moriarty, 1985). The bioaccumulation of persistent lipophilic pollutants has been observed in many bird species, and especially in raptors and fish-eating species. In fact, a large amount of data has been published on the levels of pollutants found in birds. However, only a small proportion of this information is useful for assessing the magnitude of bioaccumulation because the amounts of pollutant in the food of specimens that are analysed are usually

unknown. Given relevant information a bioaccumulation factor can be calculated as "the concentration of pollutant in the organism divided by the concentration of the pollutant in the food of that species". However, the interpretation of such factors is complicated by: a) the possibility that concentrations of residues are not directly proportional to the magnitude of the exposure; b) the difficulty of establishing exactly what has been eaten; and c) lack of information on the frequency-distribution of concentrations found in different individuals and concentration factors based on differing endpoints (such as whole body, wet weight, dry weight, lipid weight, etc.) (Moriarty, 1985).

Food is the principal source of most pollutants for birds (Moriarty, 1985), but food varies with season, age, size and geographical location. It is, therefore, difficult to determine precisely what a bird eats in the field. To predict the likelihood of bioaccumulation of a chemical, two measurements are needed: the percentage assimilation from food, and the degree of persistence within animals. Thus a detailed knowledge is required of the environmental pathways of a contaminant to indicate those species likely to get the highest exposures, together with measurements within organisms during and after exposure to assess the rates of assimilation and loss.

Early studies on pollution by organochlorine insecticides highlighted the problem of transfer along food chains and of bioaccumulation. The work on organochlorines has tended to focus on monitoring persistent pollutants to determine their distribution through certain food chains rather than studying the basic mechanisms of transfer and accumulation. Moriarty & Walker (1987) point out that the study of the uptake, metabolism, and excretion of pollutants by individual species is more useful. They suggest that such data can be used to predict the behaviour of pollutants in individuals

from which predictions can be made of the fate of pollutants in food chains. The validity of such predictions can be tested experimentally. The advantage of studying mechanisms is that this fundamental data can be used to make wide-ranging predictions about pollutant transfer and accumulation. Ultimately the data can be used to formulate predictive models (Walker, 1990b). The food chain transfer of contaminants has been widely studied in fish and invertebrates; however, the relationship between contaminant concentrations in birds and in their food is often weak and highly variable. Much of the variation probably results from the fact that birds are highly mobile and from the energetics of foraging in fluctuating environments (Lovvorn & Gillingham, 1996). A computer model was developed to take into account the variability of contaminant uptake by birds. The model was based on diving ducks, and revealed that the contaminant level of the food was less important than food dispersion and the resultant energy costs of searching for food. Hence, basing the prediction of bioaccumulation factors on a constant daily intake, or assuming that food intake simply increases as energy costs rise, are insufficient to predict properly the accumulation potential of any given contaminant.

An understanding of the bioaccumulation of chemicals by birds is vital to an assessment of the potential hazard. The basic physico-chemical properties of a chemical or chemical group (such as octanol-water partition coefficient) will provide information on the potential for accumulation. However, it is important to ascertain the actual accumulation of a pollutant, based on the mechanisms of its uptake, metabolism and loss, in order to make realistic predictions regarding the long-term effects of persistent pollutants on birds. Bioaccumulation and biomagnification of chemicals is now a central part of the risk assessment process (Franke et al., 1994); however, the interpretation of such data requires

expert judgement to extrapolate from simple physico-chemical properties to the actual accumulation, and the possibility of biomagnification, of chemicals in the field.

4.4 Biomarkers

Biomarkers are defined as biological attributes which respond to a chemical or chemicals in a way proportional to exposure. They may sometimes involve toxic effects. They would include, for example, inhibition and induction of enzymes, eggshell thinning, hormonal changes, immune responses and stress proteins. This area is a growing field with new techniques being developed all the time. Biomarkers, especially those of a non-destructive nature, are likely to play an increasingly important role in field trials, and monitoring programmes, and as general indicators of environmental harm to bird populations.

The utilization of biochemical and physiological responses in the biological assessment of the environmental impact of pollution has developed only over the last 10 to 15 years. Biomarkers make it possible to integrate the pharmacokinetic and toxicological interaction resulting from exposure to a complex mixture of chemicals in an exposed organism, providing the cumulative effect of toxicant interactions in molecular or cellular targets. They can be used to integrate different episodes of exposure in time and space and can represent rapid responses to toxicant exposure, providing an early warning signal of long-term effects (Fossi et al., 1994a). The role of biomarkers in environmental assessment has been extensively reviewed in recent years (Huggett et al., 1992; Peakall, 1992; Peakall & Walker, 1994).

Esterases are members of a large and varied group of enzymes, divided into A esterases which hydrolyse organophosphates and B esterases that are inhibited by them (see section 3.4). The biomarker most frequently used for diagnosing exposure to organophosphate or carbamate pesticides is the B esterase, cholinesterase. Most studies have involved the measurement of esterases in the brain. However, although still limited by some difficulties, the detection of esterases in serum can be conducted with a high degree of sensitivity. Both serum butyrylcholinesterase and brain acetylcholinesterase inhibition have proved useful as biomarkers in laboratory studies on the effects of single pesticides and the interactive effects of different groups of pesticide (Fossi et al., 1992; Johnston, 1995). The difficulty lies in trying to use the same techniques to interpret data from field studies. Fossi et al. (1992) found a significant correlation between serum butyrylcholinesterase and brain acetylcholinesterase in quail dosed with organophosphorus and carbamate insecticides. They concluded that the non-destructive biomarker butyrylcholinesterase can give an early semi-quantitative warning of the toxic effects of organophosphate and carbamate insecticides in birds. Swallows nesting in a barn treated with an organophosphate showed drastic reductions in both serum butyrylcholinesterase (56%) and carboxylesterase (36%) (Fossi et al., 1994b). Thompson & Walker (1994) conclude that the use of blood esterases in monitoring exposure of birds to field applications of organophosphates and carbamates can be invaluable, particularly in the interpretation of effects. However, caution must be taken in their use and interpretation, because a large number of variables can affect their activity. In addition, although laboratory studies are valuable in investigating the time course of inhibition and recovery following pesticide exposure, such data should be extrapolated to field data with great care due to the widely differing routes and time course of exposure. Walker (1992) points out that blood esterases are extremely variable, making it difficult to obtain reliable control

values. In the laboratory it would be possible to obtain pre-dosing samples for each individual, whereas in the field the problem of individual variability is difficult to overcome. When placed alongside both diurnal and seasonal variations in blood esterases, results are not easy to interpret. One way of overcoming these problems and at the same time utilising serum esterases as a non-invasive biomarker would be to use antibodies of particular esterases. Such antibodies would enable the concentration and specific activity of a particular esterase to be measured. The use of diagnostic kits for the measurement of specific activities of blood esterases would be extremely valuable in measuring the exposure of birds to both organophosphorus and carbamate insecticides. As yet, however, such kits have not been fully developed.

Many chemicals such as heavy metals and chlorinated aromatics can disturb porphyrin metabolism in birds. Chemicals can modify the activity of enzymes involved in haem biosynthesis which results in an alteration in the amount or composition of porphyrin. The pattern of porphryin accumulation in tissues, blood and excreta can be used as biomarkers of exposure. Porphyrins can be sensitively and accurately measured in relatively small samples using high-performance liquid chromatography (HPLC) with fluorescence detection (De Matteis & Lim, 1994). Fox et al. (1988) found that concentrations of highly carboxylated porphyrins in the livers of adult herring gulls from colonies throughout the Great Lakes were markedly elevated compared with gulls from coastal areas. The authors suggest that the high levels of porphyrins reflect derangement of haem biosynthesis induced by polyhalogenated aromatic hydrocarbons.

Mixed function oxidases (MFOs) play an important role in the metabolism of many lipophilic organic contaminants. The MFO system can provide indices of both exposure

and effects. The biochemical responses of the different isoforms of cytochrome P-450 are relatively specific to the compounds evoking the response and MFO is simple to detect and highly sensitive. Therefore, the MFO system is one of the most widely used biomarkers in field studies as an indicator of exposure to petroleum hydrocarbons, halogenated hydrocarbons, and polychlorinated and polybrominated biphenyls. Walker (1980) showed that there were significant species variations in hepatic microsomal enzymes that metabolize pollutants. The relationship between hepatic microsomal enzymes and pollutant exposure has been confirmed in the field (Knight & Walker, 1982). The detoxicant activities of the MFO system were found to be much higher in black-headed gulls feeding on landfills compared with those on a lagoon (Fossi et al., 1991). Fossi et al. (1995a,b) confirmed a relationship between feeding habits and evolutionary interspecific differences in MFO activity. They found that omnivorous birds with high MFO activities are better adapted and/or more adaptable to life in polluted environments. Therefore, a knowledge of different 'species-specific' detoxification abilities could be used to indicate species potentially at risk.

Two non-invasive biomarkers which could be utilized in the field are the transthyretin-retinol binding protein complex and the precursors of clotting proteins. Disturbances to the binding protein complex by PCB metabolites causes three changes which can be easily measured in plasma: reductions in the blood levels of Vitamin A and thyroxine, and a reduction in the binding of thyroxine to transthyretin. These effects have been observed in laboratory studies although the response depends on species. A field study on cormorants (*Phalacrocorax aristotelis*) indicated a reduction in plasma thyroxine but no change in Vitamin A following exposure to PCBs (Brouwer, 1991). Anticoagulant rodenticides, such as warfarin and second generation rodenticides, inhibit the Vitamin K cycle causing

an increase in the precursors of clotting proteins to appear in the blood. The development of an enzyme-linked immunosorbent assay (ELISA) utilizing antibodies raised against certain precursors of avian clotting proteins would provide a good biomarker for the secondary effects of rodenticides on birds (Walker, 1995).

Eggshell thinning by DDE and other contaminants has been recognized as a biomarker associated with impaired hatching success (see sections 3.2 and 4.2). Thinning is detected by measuring shell thickness directly or by weight per unit of length x breadth (Ratcliffe index) (Anderson & Hickey, 1972; Anderson & Hickey, 1974; Hickey & Anderson, 1968; Ratcliffe, 1967; Ratcliffe, 1970). However, more recently, the measurement of eggshell breaking strength has been developed as another sensitive index of shell thinning and structure (Carlisle et al., 1986).

Other possible biomarker techniques include clinical biochemistry, thyroid function, aminolevulinic acid dehydratase inhibition, immunotoxicology, induction of metallothionein, haemoglobin adducts, DNA alterations and stress proteins. The measurement of cellular, biochemical and macromolecular constituents in blood and the subsequent evaluation of the health of an animal is referred to as clinical biochemistry. It is a diagnostic technique which has been refined in humans and domestic animals and could be used as a field biomarker for wildlife (Fairbrother, 1994). The thyroid gland plays a central role in metabolic processes and the ability to measure subsequent hormonal changes caused by chemicals means that it is a possible biomarker. Aminolevulinic acid dehydratase (ALAD) inhibition is a sensitive dose-dependent measurement that is specific to lead (see section 3.4). The technique has been used extensively as a non-destructive

biomarker for the exposure of birds to lead in the field (Dieter et al., 1976; Scheuhammer, 1989).

The immune system can serve as a useful indicator as to the overall health of an animal. Biomarkers based on the immune system include lymphocyte mitogenesis, antibody-producing cell formation, antibody production, and non-specific macrophage activity. The induction of metallothioneins have been studied in birds in relation to metal exposure (St Louis et al., 1993), although the technique does not measure toxic effect and tends to give no advantage over chemical analysis. The detection of adducts to haemoglobin has been investigated in humans and recently as non-destructive biomarkers to assess environmental contamination. Alteration of haemoglobin has been shown to give an indirect measure of the dose that the DNA was exposed to in cells which are potential targets for genotoxic agents. DNA damage has also been proposed as a useful parameter for evaluating the genotoxic properties of environmental pollutants and would represent a biomarker based on molecular mechanisms underlying toxicity; however assays for DNA damage would involve destructive sampling procedures. Finally, stress proteins are induced by a variety of compounds and could be used as biomarkers, although it is difficult to separate the effects of chemicals from non-chemical stressors. Further research might reveal some specificity of stress proteins.

The term biomarkers thus covers a wide range of techniques many of which involve the analysis of tissues and organs such as the liver, kidney and brain. Whilst techniques involving MFO activity, porphyrins, DNA adducts and esterases have been traditionally obtained via invasive methods the main long-term benefit of using biomarkers would appear to involve those of a non-destructive nature. For birds the biological materials

obtainable by non-destructive techniques include blood and muscle biopsy, eggs, excreta and feathers. The modification of former invasive techniques and methods that have been conceived and standardized on non-invasive material such as blood chemistry, vitamin A and micronuclei mean that many non-invasive techniques are available to the researcher.

Many of the biomarker techniques, such as the measurement of inhibition of serum 'B' esterases, are only biomarkers of exposure; however, one of the most compelling reasons for using biomarkers is that they can give information on the effect of pollutants rather than mere quantification of the levels present (Peakall & Walker, 1994). Techniques such as the measurement of DNA damage, disturbances to the transthyretin-retinol binding complex and the measurement of precursors of clotting proteins all represent measures of molecular mechanisms which underlie toxicity (Walker, 1995). Biochemical biomarkers have considerable potential for measuring the effects of chemicals under field conditions, although in the selection of tests, it is important to consider the specificity of the test and the degree to which the change can be related to harm. Suites of both specific and non-specific biomarkers in carefully selected combinations have the potential to play an important role in pollutant assessment.

5. Hazard & Risk Assessment

There are many schemes used for the hazard and risk assessment of toxic chemicals and pesticides. Thirteen of these schemes were reviewed recently for an OECD workshop on environmental hazard and risk assessment (OECD, 1995). All such schemes included requirements for aquatic toxicity data, such as tests on algae, daphnia and fish, and many also required the use of terrestrial data, such as plant and earthworm toxicity tests. I shall concentrate here on those schemes which explicitly require the use of bird toxicity data. All of the schemes concerned with the terrestrial environment have either a formal or informal tiered structure. Tier 1 represents the first point in an assessment at which exposure and effects are compared. The differences in the number of tiers beyond this point depends on the characteristics of the particular risk assessment scheme being used. The final assessment involves an iterative procedure involving progressive refinement of exposure/effect ratios.

Bird toxicity data are only used to a limited extent in most assessments of general chemicals. The OECD aquatic effects assessment for general chemicals is intended for determining effect levels only and does not deal with the estimation of exposure or with the combination of exposure and effects to derive a risk assessment. The scheme only addresses the aquatic compartment but includes sediments and an assessment of secondary poisoning of fish-eating birds and mammals. Data from at least three bird/mammal species are preferred for the assessment of secondary poisoning effects. The toxicity data are used to estimate an MPC (maximum predicted concentration) in water that will cause no risk to 95% of species in the aquatic ecosystem and will not allow bioconcentration in fish to a level that would exceed the No Observed Effect Level (NOEL) for fish-eating

birds and mammals. Extrapolations are used from single species toxicity test results to a concentration that supposedly protects the aquatic ecosystem. These extrapolation factors (otherwise known as "assessment" or uncertainty factors) are used in schemes, and vary in magnitude according to the data available and to the predictive uncertainty of the test results. For example, a large factor would be applied to simple acute toxicity tests, but this could be reduced if longer term tests give reassurance, and data from full-scale field studies might reduce it even further.

The original MPC methodology did not take into account secondary poisoning, but Luttik et al. (1993) proposed a general algorithm for predicting its likelihood in birds. The algorithm was produced by analysing an aquatic food chain and a terrestrial food chain. MPCs are generated as follows:

i) aquatic food chain

$$MPC_{water} = NOEC_{bird}/BCF_{fish}$$

ii) terrestrial food chain

$$MPC_{soil} = NOEC_{bird}/BCF_{earthworm}$$

(NOEC = No-Observed-Effect-Concentration; BCF = Bioconcentration Factor)

The authors proposed further developments, by increasing the complexity of the food chains and incorporating other factors, such as differences between metabolic rate in the laboratory and field, the bioavailability of a compound, and processes such as migration and breeding time. Romijn et al. (1994) analysed the proposed algorithm specifically for terrestrial food chains. They found important differences between BCFs for terrestrial pathways compared with aquatic ones. BCFs for earthworms were more dependent on

soil-related properties than on compound-related properties. It was concluded that the algorithm for terrestrial food chains can only be applied in clearly defined situations. In addition, because of the difference in food consumption and energy budgets between laboratory and wild birds, a correction factor needs to be derived (Everts et al., 1993). The factor that was proposed includes the difference in energy content between grain fodder (laboratory) and natural food (field) and in energy expenditure between laboratory and field. The NOECs corrected by this factor can then be used for the estimation of MPCs in food.

A similar scheme to that utilized by OECD is now employed by the European Community for both new and existing chemicals. However, this scheme uses a parallel calculation of the predicted exposure concentration (PEC), as well as a predicted no effect concentration (PNEC). If there are insufficient monitoring data, the PEC can be calculated using modelling data. As with the OECD scheme, bird toxicity data are used only to generate estimates of the risk of secondary poisoning. The final PNEC is based on toxicity data and the application of the "assessment" factor (or uncertainty factor). The final estimate of risk is based on the ratio between the PEC and the PNEC. Risk values categorized from the PEC/PNEC ratio lead to one of the following: a) a need for further tests or information, b) no need at present for further investigation or c) a need for risk reduction beyond measures already in place. The triggers to move from one tier to another with further testing are thus based entirely on values of PEC/PNEC.

The U.S. scheme under the Toxic Substances Control Act (TSCA) assesses the risks of both old and new industrial chemicals, as well as of genetically engineered micro-organisms for the purposes of notification. Unlike the previous schemes, this utilizes all

types of bird toxicity data in an attempt to quantify the risk to birds. Risk is assessed in tests at the individual level but the potential impact at the population, community and ecosystem levels are also considered (through extrapolation models), as is the potential for bioaccumulation. Severity of risk is determined by a quotient method in which the predicted environmental concentration (PEC) is divided by a toxicity endpoint (similar to the EC methods). The scheme is tiered such that simple data are required first, followed by more complex data at higher tiers. Tier I includes acute (oral) toxicity data, tier II additional sub-acute dietary toxicity data, tier III reproductive toxicity tests and tier IV field testing. Assessment factors are applied to the effects data and these would be reduced at each successive tier.

The Uniform System for the Evaluation of Substances (USES) was developed in the Netherlands for assessing human and ecological risks posed by new and existing substances, pesticides and biocides. It is a multiple computer program-based system intended to support decisions of governments and industries regarding priority setting, data requirements and hazard/risk assessment and management. It incorporates many commonly used fate models. It is a tiered scheme used for both initial and 'refined' risk assessments by comparing the PEC with No Effect Concentrations (NEC). Environmental exposure scenarios used include terrestrial ecosystems, predators (fish/worm-eating birds) and specific terrestrial organisms residing in treated areas for pesticides. Effects assessment incorporates data from both acute and dietary toxicity testing with birds, and the use of NOEC from dietary tests. If European production exceeds 100 tons per year, avian reproductive toxicity data are required. This is an arbitrary figure derived politically rather than on a scientific basis. The scheme uses assessment factors which vary according to the type of data available. The USES scheme,

like the EC and OECD, relies on models rather than demanding further testing or monitoring.

It is not surprising that the schemes which have incorporated the most detailed information regarding bird toxicity testing and exposure are those specifically designed for assessing the impacts of pesticides. OECD organized a workshop on ecological effects assessment in 1988. For the terrestrial environment, a generalized scheme was developed to outline the hazard assessment process for evaluating a pesticide's effect on birds and mammals. Data on use, product chemistry, environmental chemistry, mammalian toxicity and ecotoxicology should be collected for the initial assessment. Exposure data would include water solubility, octanol/water partition coefficient, hydrolysis, photolysis, application rate, frequency and site. Ecological effects information would include acute oral and subacute dietary toxicity. The initial assessment would establish whether the chemical is persistent or bioaccumulates. If there is a comfortable margin of safety, then no further testing is required. Otherwise further testing or data acquisition is required. To address the question of reproductive effects, laboratory tests are needed. In order to address lethality, two approaches could be taken: the predicted exposure level could be refined by modelling or by field residue monitoring, or further toxicology tests could be performed. If there is still a concern with a particular pesticide, a second round of assessment would take place to give a new quotient (NOEL/PEC). If further data are required, then the third process would most likely be field testing of the product to examine exposure and effects (OECD, 1989).

Greig-Smith (1992) identified several critical issues in the design of risk assessment procedures for predicting the ecological impact of pesticides. Flexibility in the choice of

test protocols means that data can be taken from many sources and allows for technical advances; however it may be difficult to compare different protocols. A possible starting point for regulatory assessment is to assume that a proposed pesticide will cause ecological damage so that testing can be directed. Predictions can be based on key studies and exposure-related studies, extrapolation between species and conditions, the use of trigger values in stepwise testing, and the setting of margins of safety and uncertainty analysis. Expert judgment allows flexibility and can increase the accuracy of the assessment. The classification of risk recognizes the variability in data and allows comparisons across diverse aspects of concern. All assessment schemes should be validated to improve confidence in the decision-making process and to improve future assessments. Many of the principles outlined above have now been incorporated into assessment schemes used for pesticides and it is important that all of these issues should be borne in mind whenever assessment schemes are being developed or modified.

The U.S. Environmental Protection Agency (EPA) ecological risk assessment schemes under the Federal Insecticide, Fungicide and Rodenticide Act (FIFRA) are designed for the purposes of registration of pesticides. The schemes originated from the early 1980s and have been revised on several occasions. The main concern of the schemes, stated in the 1986 versions, was the mortality of non-target organisms including endangered and threatened species. The schemes initially covered single species populations, but have since been widened to cover communities and ecosystems. The schemes deal with toxicological (effects) and environmental exposure separately and then compare them to assess risk. A tiered approach is used and can include avian mortality, reproductive effects testing and field studies. Expert judgment is used at a number of places within the schemes, such as natural history characteristics of non-target organisms and effects on

endangered species. In 1992 the U.S. EPA implemented a 'new paradigm' for ecological risk assessment and identified three phases of the process: problem formulation, risk analysis and risk characterization. In the 'new paradigm', higher tiered ecological issues are more commonly resolved by drawing upon modelling, environmental characterization and monitoring, rather than by field toxicity testing. The framework is currently under review and new guidelines have been drafted which expand the discussion of problem formulation, analysis, and risk characterization and further explore the interaction between risk assessors and risk managers.

The EPPO/CoE (European and Mediterranean Plant Protection Organization/Council of Europe) decision-making scheme is also intended for the environmental risk assessment of agricultural pesticides. It is a questionnaire based scheme divided into a number of distinct subschemes that assess risks in different environmental compartments. A tiered approach is used that requires more detailed data at higher levels to derive an ultimately quantitative risk assessment. The scheme requires data from a broad range of taxonomic groups, including two bird species (acute oral toxicity; one species only for dietary toxicity and extra data on irritancy and reproduction only if available). There are many different triggers to move from tier to tier in the sequential scheme. Measures taking into account exposure (the concentration of active ingredient in the environment) and effects (often a measure of toxicity) are considered to be the ultimate trigger in deciding risk in this scheme. Risk thresholds can be derived from the Toxicity:Exposure ratio (TER). For vertebrates, the high risk threshold is 0.1 for LD_{50}/LC_{50} derived figures and 1 for NOEC/NOEL derived figures. The low risk threshold is 0.01 for LD_{50}/LC_{50} derived figures and 0.1 for NOEC/NOEL derived figures.

The U.K. pesticide registration scheme is based around the EPPO/CoE scheme and the risks to birds section of this scheme will be reviewed here as an example. Bird toxicity data are summarized, providing LD/LC_{50} doses at which effects were seen and the time period over which these occurred, and NOEL/NOECs; the need for further testing is highlighted. The use of the pesticide is considered in terms of exposure; for example, molluscicide baits are most likely to lead to exposure of pheasant and sparrows, poisoned earthworms to lapwing, gulls and thrushes, and sprays on arable crops to grazing pigeons and geese etc. The Toxicity:Exposure ratio (TER) (≡ a PEC/PNEC ratio) is calculated. For LD_{50} data, exposure is calculated in terms of lethal dose given orally whilst for LC_{50} data, the effect and lethal levels in diet are compared with residues in the food source. Residues on seed are calculated from the amount of active ingredient applied per hectare using seed loading and the sowing rate.

Information on food consumption by different bird species is utilized in the assessment and, by using daily food intake information, it can be estimated whether a lethal dose could be obtained from consumption of treated food. For secondary poisoning, information is needed on residues in poisoned earthworms or rodents. Other questions are also taken into account such as: will the time of year or crop growth stage of use reduce exposure?, is the use regional, so affecting exposure of regionally-restricted species?, and is exposure other than acute, for example repeat sprays during the breeding season? If a hazard is identified, then data requirements are set to answer the specific questions raised. Further laboratory studies may be required, such as palatability studies, field studies or surveillance. Data on reproductive effects in birds are also reviewed and concerns can be dealt with under the scheme (HSE/PSD, 1995). The scheme is, therefore, a stepwise

decision tree in contrast to the fixed tiers of risk assessment for new and existing industrial chemicals.

The OECD workshop on hazard/risk assessment in 1994 identified several areas of improvement with regard to terrestrial assessments: improving and refining the use of assessment factors and improving the understanding of the ecological significance of effects seen in the field on populations and ecosystems. The importance of expert judgement at all stages of assessment was identified, as was the continuous validation of the assessment scheme, and establishing criteria for the validation of non-standard data. Overall the working group on terrestrial effects assessment felt that both general chemicals and pesticides should be accommodated in a common concept and risk assessment framework (OECD, 1995).

Progress in the field of environmental hazard and risk assessment, especially involving terrestrial ecosystems, requires inputs from a wide variety of disciplines. It is clear that, as the field develops, interdisciplinary cooperation is critical in providing the complex data sets required. Recent reports and publications show how information from disciplines which hitherto have not been directly involved in ecological assessment are now being utilized. Dobson et al. (1995) have developed a blueprint to assess the risk to birds of industrial chemicals released into the terrestrial environment. The scheme is an extension of that proposed for pesticides by EPPO/CoE. For all British bird species, body weight, food consumption and components of the diet have been identified and modelled. A methodology for calculating estimated toxicity to all species based on published toxicity tests is presented. For each species a Toxicity:Exposure ratio (TER) can be calculated as an indicator of risk. British bird species associated with U.K. Land Cover classes have

been identified. The methodology forms the possible basis for site-specific risk assessment of emitted substances. Similarly, Geographical Information Systems (GIS) have been used to perform analysis of the spatial relationships between crop and non-target areas since Crabtree et al. (1994) introduced the use of such techniques to environmental risk assessment. McGaughey et al. (1996) identified that the weakest link in the prediction of risk is in the interpretation of a given local event or condition with respect to its meaning for a broad geographic area. The authors suggested that Geographical Information Systems (GIS) which analyse data while maintaining their spatial relationships, and remotely sensed data, which define land cover variables, provide 'real world' characterization of the environment.

Ultimately decisions regarding the regulation of chemicals have to be made from the battery of tests, field trials, monitoring and incident investigation. Hazard identification involves the identification of the substances of concern, their adverse effects, target populations and conditions of exposure. When the hazards are combined with the probability and the actual exposure, a risk assessment can be made. Risk assessment is becoming central to the decision-making and priority-setting of both national governments and international agencies. However, such assessments are only as good as the information on which they are based. It is, therefore, essential that all aspects of predictive testing and field validation are continually monitored and revised. There are many sources of errors at all stages of the risk assessment procedure, and evaluations and conclusions can be subject to considerable uncertainty. It is, therefore, important that reporting of assessments is carried out in a transparent manner so that the degree of uncertainty is made clear. For risk assessments to form the basis of predicting chemical effects on birds, it is necessary for the schemes employed to be relatively flexible. Such flexibility would

enable the assessment to be related to actual exposure scenarios rather than to the simple generation of standard data sets. Assessments should involve input from a variety of disciplines. At all stages of risk assessment there should be the ability to use expert judgement.

Most studies concerned with the hazards of pesticides to birds involve an assessment of the effects of individual compounds. However, under field conditions, birds may be exposed to a wide range of agricultural chemicals which may interact in such a way as to increase or decrease the toxicity of any one. Interactions between pesticides, and the mechanisms responsible for them, can be studied in the laboratory, but again the results from such studies can seldom be directly extrapolated to the field. If potential synergisms were to be routinely assessed, in addition to single products, there would be major implications for registration. The way forward may well be exposure scenarios based on particular mixtures rather than trying to use independent assessments to predict the consequences.

6. Discussion & Conclusions

6.1 Discussion

The effects of chemicals on birds has created a great deal of public concern and scientific debate. The majority of bird species are fairly conspicuous and relatively easy to observe. They are abundant, visible, colourful, inhabit a diverse variety of habitats and overall have great aesthetic appeal. The popularity of birds means that they have been studied in unrivalled detail by conservation groups, national and international organizations, and research institutes. The wealth of information which has been gathered regarding species distribution, breeding numbers and subsequent population changes has provided a good indication of environmental problems. However, it is in using this information for predictive purposes, with regard to chemical impacts, where much of the difficulty lies. Laboratory toxicity testing is mostly carried out as a routine requirement of both national and international regulatory authorities, and the prediction of effects is still centred around these standard tests. However, major problems have been identified with regard to laboratory testing systems, including laboratory to field extrapolation, statistical robustness, species and age-related sensitivity. Most laboratory studies are carried out on unrepresentative species under unrealistic conditions.

It is generally agreed that the acute oral toxicity test is necessary as part of the first tier of any ecological risk assessment scheme. The test can be used to compare in a consistent manner the acute toxicity of different chemicals. However, a single LD_{50} value does not provide sufficient information on the acute toxicity of a chemical, and for comparing chemicals, it is also necessary to study the slope of the toxicity curve as well as its asymptotes. For those substances which are not expected to be toxic, a single limit test at

an upper limit dose should be adequate. If there is uncertainty in the risk assessment, and more information on inter-species differences are considered helpful the Approximate Lethal Dose could be determined on further species using an Up and Down method. Both the single limit dose and the Approximate Lethal Dose have the advantage of using fewer test animals. The need to limit the number of birds used within the testing regimes must always be of prime concern, as long as this does not compromise the statistical robustness of the tests. However, some still argue that the acute oral test is not relevant to environmental exposure and risk assessments, and that short-term dietary exposure is more realistic.

While the sub-acute dietary toxicity test does appear to be more 'environmentally realistic' than the acute test, and could offer some insight into the chronic toxicity and repellency of a chemical, it is still a simple bioassay for lethality, with severe limitations. In particular, problems could arise from the test conditions employed and their reproducibility. Some have recommended that the test is increased from 5 to 21 days to provide more indication of a pesticide's potential to cause chronic or cumulative toxicity. On the matter of repellency the potential for avoidance could be examined within the testing regime but the difficulty is how to do this. Laboratory tests for repellency do not usually reflect the reality of the field situation. There is some merit in modifying the dietary test to include choice between undosed and dosed food, which would reduce the problem of repellency leading to false results caused by starvation rather than toxicity. Whether this would be sufficient to study the effects of chemicals in which an aspect of repellency is intrinsic to its usefulness as a pesticide remains to be seen. It might, however, be possible to use the dietary test as a screening exercise for repellency, and to follow it up with more sophisticated testing for repellency on specific chemicals. Distinguishing between

repellency and direct toxicity is obviously vital for the interpretation of test results. Both the acute and short-term dietary toxicity tests are based around death as the endpoint and, therefore, are insufficient to meet all the needs of predictive testing, which should also include sublethal and long-term effects (as apparent with such chemicals as the organochlorine insecticides, organometals, dioxins and polychlorinated biphenyls).

The reproduction test was introduced to tackle one aspect of the problem of predicting sublethal effects of chemicals. It appears to be the most 'sophisticated' of the standard tests; but its critics point out that even this test implicitly assumes a very simple model for the control of reproduction by environmental variables and that it is still firmly founded on human health assessments. Like the acute and dietary tests, the reproduction test has its problems, but with some modifications it could still provide a good indication of the direct effects of chemicals on reproductive physiology. Recommendations for modification include studies on the relative sensitivity of species, use of biomarkers, refinement of statistical procedures, development of a short term exposure reproduction test and field validation of the test. Even so, if all of these modifications are enacted, the test could still tend to be a poor indicator of population effects in the field, because both species and conditions are inappropriate. It has been suggested that a breeding population of songbirds in an aviary with no interference through sampling, and with natural incubation and social interaction similar to that found in the wild would be a better indicator of overall reproductive success. A recommendation for using a full breeding cycle using passerine species has been put forward. The reproduction test is, therefore, useful for indicating potential sublethal effects of chemicals, but is not a realistic model of the field situation.

Many non-standard tests have examined a wide variety of behavioural and reproductive effects and endpoints. In theory, behavioural responses could provide a link between the biochemical and ecological consequences of environmental contamination and, therefore, give a measure of the effect of a chemical in the field. In practice, however, most non-standard tests would be expensive to run on a regular basis and often present difficulties of interpretation. None-the-less, the information continually gathered from such tests is useful background for standard testing procedures. For example, biochemical assays that have been developed are gradually becoming an integral part of both laboratory and field testing programmes. There are also circumstances where 'one-off' non-standard tests are the only feasible approach to a particular problem.

The laboratory tests that are available do at least provide the possibility of screening chemicals for potential effects as long as their limitations are understood. Public resistance to large-scale animal testing could well preclude the future generation of large data bases on lethal toxicity; this will challenge scientists to develop effective methods of predicting hazards to wildlife using minimum numbers of animal subjects. The best way forward in realizing the maximum potential of laboratory testing appears to be the establishment of multidisciplinary test systems. These should give a greater understanding of the way in which environmental variables or the behaviour and physiology of the test animal influence the test regime. Only with such knowledge can the real strengths and limitations of laboratory toxicology be evaluated. Then, and only then, can data generated from laboratory testing be readily used in the assessment process. It will probably be impossible ever to design a system of tests to assess all conceivable hazards to communities, and there will always be limitations to the predictive capacity of laboratory-based studies. It will

always be necessary therefore to use data generated in field trials and monitoring to refine the laboratory-generated data.

Field studies take many forms, ranging from semi-field trials, with caged birds in sprayed areas, to full-scale experiments extending over many sites. The advantages of utilizing field studies in risk assessment schemes include natural conditions, wide range of species and realistic exposures. The disadvantages usually centre around the statistical usefulness of the results. Field trials still rely heavily on corpse counting, but biochemical and immunochemical assays open the way for more sophisticated monitoring in the future. Good field trials are vital to the refinement of the risk assessment process, as are monitoring programmes, for without such feedback, predictions and models will never be put to the test. The development of more sensitive biomarkers is crucial to effective future monitoring. To predict with greater confidence the 'real' field effects of chemicals released into the environment requires a knowledge of how bird populations are affected above and beyond natural constraints. Much development work has concentrated on test regimes and field validation, but yet little attention has been focused on the interpretation of the data, in the light of the other factors which influence the survival of wild birds. As has been stated several times, the prediction of chemical effects on birds requires an integration of many diverse scientific disciplines. Then, rather than relying on laboratory test results only, the field data could provide the basis for developing models of how communities of animals and plants interact, enabling the wider environmental effects of chemicals to be assessed.

Concerns about the effects of chemicals on birds have changed over the last 30 years. The 1960s and early 1970s were dominated by the highly persistent organochlorines and their

devastating effects on bird of prey populations. Many of these chemicals have now been replaced by organophosphates, carbamates and synthetic pyrethroids, and risk assessments on these chemicals must now focus more on short-term impacts and the ability of populations to recover from sporadic mortality. However, some organochlorines are still used widely in some other parts of the world, chiefly in the tropics. Prior to the 1980s, most ecological risk assessments involving the newer classes of chemicals employed primarily laboratory work through a tiered framework proceeding from LD_{50} and LC_{50} tests to reproductive toxicity tests. Since then, the ecological impact of chemicals, and especially pesticides, has come under even greater scrutiny. The 1980s saw wildlife toxicology and associated ecological risk assessment schemes evolve, from a relatively primitive protocol into a rapidly growing area of science. The past few years has seen growing pressure from the public to answer questions concerning potential chemical hazards to the environment. Recommendations and regulations for extensive pre-registration and re-evaluation testing are now in place for those pesticides likely to pose environmental hazards. Although quantitative ecological risk assessments are becoming ever more sophisticated, there is still a long way to go to utilize fully all the data available. Many of the risk assessment schemes for pesticides are centred around the Toxicity:Exposure ratio (TER) which relies heavily on the data generated from laboratory-based toxicity tests. The limitations of such tests have already been outlined and bring into question the predictive usefulness of the TER. Nevertheless the TER is still useful as an indicator of risk if weight of evidence and professional judgement are applied to the process, and as long as the final risk assessment is carried out at the local level incorporating all relevant information from a variety of disciplines. Site specific exposure data and relevant species are vital to a realistic risk assessment.

Future developments in risk assessment will probably emphasize the indirect effects of chemicals, and especially pesticides, such as depletion of prey, removal of competitors and alteration of habitat. This will be no easier than predicting direct effects, and the same limitations on field studies apply. It is always difficult to identify the real causes of population declines especially by short-term study. Much information is available on changes in bird numbers, but there is a limited understanding to what extent chemicals influence these changes, except for a few species, such as grey partridge and sparrowhawk discussed above. To successfully predict direct or indirect effects of chemicals on birds requires substantial background knowledge of those bird species that might be affected. Thus although many species of farmland birds in Britain are fast declining, the causal factors have proved extremely difficult to pinpoint in the absence of detailed studies. Birds clearly have a role to play as indicators of environmental health, and are highly valued by a large proportion of the voting public. The values attached to bird species and populations will always have to be weighed against the benefits of chemical use. Such considerations are, and will always be, important for the control of chemicals but move beyond the scope of this thesis.

6.2 Conclusions

- Laboratory testing has an important place in the prediction of the effects of chemicals on wild bird populations.

- Simple tests must be seen merely as a means of screening chemicals for potential effects rather than as realistic models of the real world.

- Risk assessment of chemicals should be fate-led to minimize the unnecessary use of laboratory animals. It is more efficient in resources to identify the distribution and availability of chemicals in the environment before testing for effects; otherwise substantial numbers of test animals could die assessing the toxicity of chemicals to which they will never be exposed in the wild.

- Testing should be on appropriate species with the appropriate route of exposure based on conclusions on fate.

- Test design should be varied and flexible to allow relevant risk assessment.

- Validation of preliminary risk assessment conclusions should be carried out in the field.

- Multidisciplinary studies of testing, field trials, monitoring and risk assessment should be carried out; disciplines such as toxicology, environmental chemistry, ecology, population dynamics, ethology, physiology and land use geography all have a part to play.

- Risk assessment should be flexible and include significant scope for expert judgement.

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