Methods for Estimating Risk of Chemical Injury: Human and Non-human Biota and Ecosystems
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Extrapolation from Single Species Studies to Populations, Communities and Ecosystems

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ABSTRACT

The extrapolation of ecotoxicological data is examined under three main headings, i.e., species-to-species, individual-to-population and laboratory-to-field extrapolation, in order to assess the degree of risk involved. While species-to-species variation can be large, the distribution of LC_{50} values tends to follow a positively skewed normal curve. With increasing data sets the uncertainties of extrapolation can be defined with increasing precision. The ratio, concentration of lethal to sublethal effects, such as physiological and behavioural effects, for a given species tend to be within an order of magnitude if exposure conditions are comparable. The basis for extrapolation from tests carried out on a limited number of individuals to the effects on populations is tenuous. The relationship of laboratory tests to field experiments is examined for two well-studied environmental problems: eggshell thinning in raptoral birds and the reproductive failure of fish-eating birds on the Great lakes.

1 INTRODUCTION

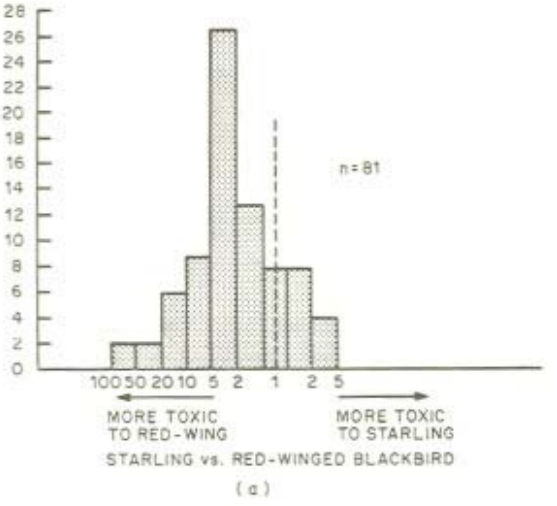
The approach being taken to this extremely complex problem is to examine several extrapolations involved singly and then to take a preliminary look at the overall extrapolation from single species tests to real world problems. The initial extrapolations are considered under three headings, namely species-to-species extrapolations, individual-to-population extrapolations, and laboratory-to-field extrapolations. The term extrapolation is not used in a strict mathematical sense but rather to describe to what extent the results of one test are valid under different conditions.

2 SPECIES-TO-SPECIES EXTRAPOLATION

The necessity for species-to-species extrapolation in assessing the impact of toxic chemicals is obvious. While the estimates of the total number of species living in the world today vary considerably, there is agreement that it is of the order of one to three million. There are innumerable examples of problems involved in extrapolation but a single one will have to suffice. Macek and McAllister (1970) measured the LC_{50} of four organophosphate insecticides for twelve species of fish. The range of the acute lethal concentrations for two pesticides (methyl parathion and Baytex^R) was from one to four, whereas for malathion the ratios of LC_{50} values were over one hundred for two species; for guthion four ratios were over one hundred, three over five hundred and one over one thousand. That extrapolation is necessary, is obvious; the problem is to assess the uncertainties involved.

2.1 Acute Lethality

One of the tests routinely carried out on new chemicals is the rat oral LD_{50}. A comparison of the oral LD_{50} values for the rat and a bird (the starling, *Sturnus vulgaris*), based on the work of Schafer (1972), is shown in Figure 1a. It can be seen from the data that the distribution of LD_{50} ratios is skewed and that the starling can be up to 500 times more sensitive than the rat. However, the starling is, on the average, roughly five times more sensitive to acute oral poisoning than the rat. The second point is that only five out of 71 chemicals fall outside two orders of magnitude.



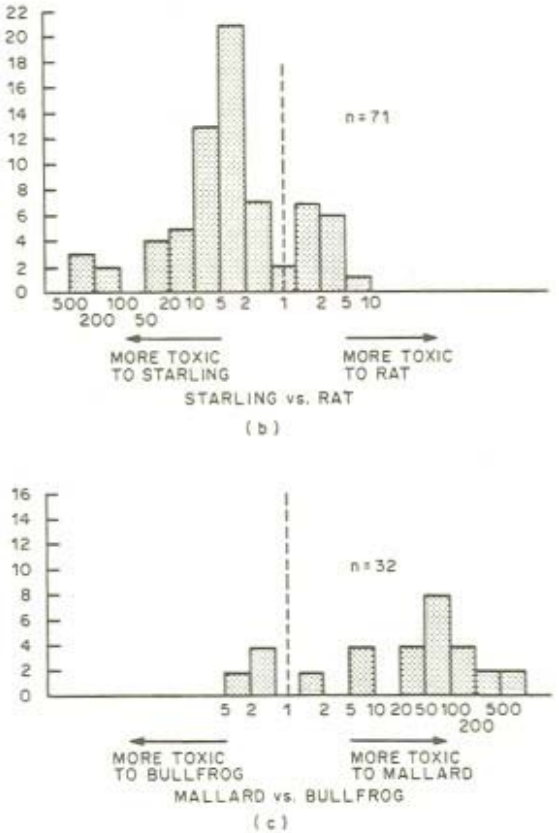


Figure 1 Species-to-species comparison of LC_{50} values. 1a. Red-winged blackbird to starling. 1b. Starling to rat. 1c. Mallard to bullfrog

Using the same data base, the starling can be compared with another avian passerine species, the red-winged blackbird (*Agelaius phoeniceus*). Again there is a clear skewing of the data (Figure 1b); the red-winged blackbird is appreciably more sensitive than the starling. However, most of the ratios fall within one order of magnitude and all fall within two orders of magnitude. As would be expected, the differences increase as the phylogenetic difference increases. Using a rather small data base, a comparison of the acute lethal levels for the mallard (*Anas platyrhynchos*) and the bull frog (*Rana catesbeiana*) (Figure 1c) based on Tucker and Crabtree (1970), shows little predictive value with four out of 16 values falling outside two orders of magnitude.

Tucker and Haegele (1971) compared the oral toxicity of 16 pesticides with six commonly tested avian species. In this instance the determinations were made

under strict protocols which reduced variability. As a result it was concluded that, given LD_{50} for any one avian species, only one time in ten would LD_{50} of the second untested species be less than one-tenth that of the first. Further, half the time LD_{50} for the second species would be twice that of the first.

Recently (Tavitz and Skumick, 1980) have shown that, for a large number of chemicals, the distribution of LC_{50} values in many aquatic species tends to take the shape of a positively skewed normal curve. Thus, although the difference from the most sensitive species to the median sensitive species may be large, a greater difference exists between the median degree of sensitivity and the least sensitive species.

Crosby *et al.* (1966) examining the wide extrapolation from *Daphnia magna* to the rat concluded that, while physiological, anatomical and biochemical differences precluded any simple, direct interspecies relation of responses, they found no instance in which a substance showed significant acute toxicity to mammals that was not also highly toxic to *Daphnia*. The converse, however, was not true.

2.2 Life-cycle Tests

Correlations between chronic toxicity values for a variety of chemicals for *Daphnia magna* and the fathead minnow (*Pimephales promelas*) were examined by Maki (1979). Using linear regression analysis he found a high degree of correlation for detergents, metals and PCB isomers and a rather poor correlation for pesticide formulations. He concluded that 'the correlation between *Daphnia* and fish toxicity values also serves to demonstrate that aquatic safety data developed for the protection of one trophic level may similarly extend to include representatives of a higher level'. Plotting the data presented by Maki as ratios (Figure 2) one finds that over one-third of the cases fall outside one order of magnitude and three cases (12 %) outside two orders of magnitude. This suggests that the uncertainties of extrapolation are considerable. Of interest is that in all three cases which fall outside two orders of magnitude, the chemicals are more toxic to *Daphnia*, supporting the earlier conclusion of Crosby *et al.* (1966) that *Daphnia* are good as a warning system.

A comparison of the Maximum Acceptable Toxicant Concentration (MATC) based on survival and effects on growth and reproduction of several species for a few chemicals has been made in a series of reports by Macek *et al.* (1968a,b,c). These data are shown in Table 1. The degree of variation is surprisingly small between fish and invertebrates.

More recent compilations of MATC values for a variety of fish species and daphnids were published by Kenaga (1979) in which he concluded 'there is no consistent pattern of MATC . . . values among all chemicals and species' and that '. . . except for closely related organisms or chemicals, the MATC should preferably be derived experimentally using *Daphnia* life cycles or fish critical life

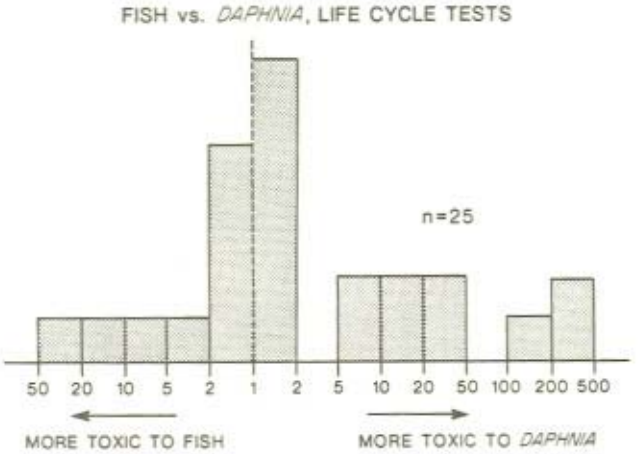


Figure 2 Species-to-species comparison of life-cycle test values; fish *vs Daphnia*

stages, partial life-cycle tests . . . ' . There seemed to be one exception to this conclusion in Kenaga's paper where he calculated the correlation between *Daphnia* and fathead minnow MATCs and found an r value of 0.76 for a variety of chemical structures. He found an even better r value (0.98) with closely related chemical structures.

One of the problems with use of comparative toxicology statistics published to date has been due to the approach of computing median estimates of unknown datum B, given experimental datum A. The estimate for datum B will be too high in half the cases. No regulatory official wishes to accept 50% odds of underestimating the risk. We suggest that a very useful estimate of MATC in untested species could be obtained from real MATC data for species A; one could statistically derive an estimate of the MATC for species B which would only be in error on the high side, say, 5% of the time.

2.3 Physiological Alterations

The effects of chemicals can be found at various organizational levels: molecular, cellular, tissue, organ, whole animal. In general, the degree of phylogenetic variation increases going from the molecular to the whole animal while at the same time increasing the applicability to the real world situation. Studies on the effect of chemicals on DNA could have relevance throughout the plant and animal kingdoms, whereas effects on thermoregulation would apply only to birds and mammals. One of the widest phylogenetic extrapolations is the use of the *Salmonella* strain of bacteria to assess the mutagenic potential of chemicals as a screen for human health effects (Ames, 1979). While this type of screening has

Table 1 Application factor* calculated for selected aquatic invertebrates and fish for six chemicals (Macek *et al.*, 1968 a,b,c)

	Atrazine	Lindane	Acrolein	Heptachlor	Endosulfan	Trifluralin
Midges (*Chironomus tentans*)	4.3–9.1	0.20–0.45				
Water-fleas (*Daphnia magna*)	4.0–7.1	0.05–0.09	0.03–0.06	0.04–0.08	0.14–0.37	0.14–0.41
Scad (*Gammarus fascietus*)	7.1–16.7	0.12–0.23				
Bluegill (*Lepomis macrochirus*)	2.0–11.1	0.08–0.11				
Fathead Minnow (*Pimephales promelas*)	1.9–4.8	0.04–0.11	0.02–0.09	0.54–1.16	0.2–0.4	0.20–0.52
Brook-trout (*Salvelinus fontinalis*)	8.3–16.7	0.06–0.12				

* Application factor is defined as Maximum Acceptable Toxicant Concentration divided by LC_{50}.

Table 2 Relative activity of mixed function oxidase activity in several classes of organisms

	Aldrin epoxidation[1]	Azo reductase[2]	Nitro reductase[2]
Mammal	1	1	1
Bird	1.28	1.20	0.52
Reptile	—	0.24; 0.77	0.06; 0.21
Amphibia	—	0.23; 0.20; 0.27	—
Fish	0.71	0.11–0.13	0.24; 0.31
Insect	0.36	—	—

[1] Chan *et al.*, 1967.
[2] Adamson *et al.*, 1965.

obvious implications for human health, its importance to environmental problems, where one is concerned with populations rather than individuals, has not been established.

Hepatic microsomal enzyme systems have been used to examine for the effects of a wide variety of xenobiotics since the first discovery of their induction by chlordane (Hart *et al.*, 1963). Induction of mixed function oxidase (MFO) enzymes is a defence mechanism to render foreign lipid-soluble materials more polar and hence more readily excreted. There is no clear link between the degree of induction and any adverse physiological effect. The variation of the activity of some of these enzymes over a range of classes of animals is shown in Table 2. In general, the activities are highest in mammals and birds and decrease in lower forms. Walker (1978, 1980) reviewed the relative activities of epoxide hydratase for vertebrates and concluded that the degree of enzyme activity closely followed phylogenetic classification, i.e., mammals > birds and amphibia > fish.

Walker (1978) found an inverse correlation between mono-oxygenase activities and the half-life of xenobiotics metabolized by this system over a range of vertebrate species. He considered that environmental studies would be aided by this approach since it would allow for the identification of species deficient in MFO activity which could be at elevated risk due to decreased ability to excrete toxicants.

3 INDIVIDUAL-TO-POPULATION EXTRAPOLATIONS

In extrapolation from single species test results to expected ecosystem effects, one variable to consider is the ratio between exposure levels which cause lethal and sublethal effects. Though an exposure may be sublethal, it could lead to population reduction if the escape behaviour from predators or reproduction are affected, for example. Tucker and Leitzke (1979) argued that sublethal effects are unlikely to occur at less than one-sixth of the exposure level which caused lethal

effects. This hypothesis tends to hold if one eliminates from comparisons all variables (such as duration of exposure) except endpoints being considered.

3.1 Extrapolation from Acute Toxicity to Life-cycle Tests

The simplest extrapolation of acute test results to life-cycle tests is to increase the time axis. A plot of survival time against concentration of DDT for rainbow trout, *Salmo gairdneri*, is shown in Figure 3 (Abram, 1967). This type of plot can be used to extrapolate to infinite time and thus gives a minimum figure for the effect of chronic lethal exposure.

For populations it may be of more interest to look at mortalities other than the standard 50% used in LD_{50} and LC_{50} estimates. The interrelationship between survival time, concentration and mortality is shown diagrammatically in Figure 4. The mortality that causes significant population effects varies greatly, depending on such factors as reproductive potential, age at first breeding, etc. The time

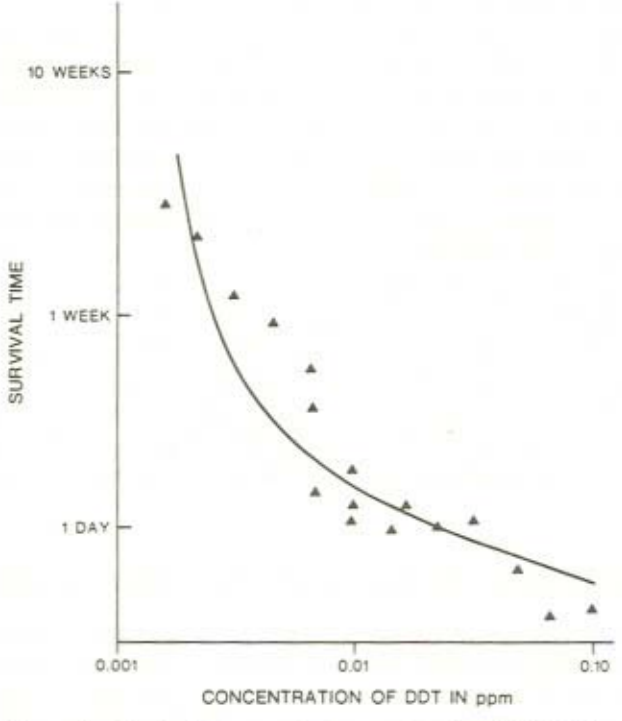
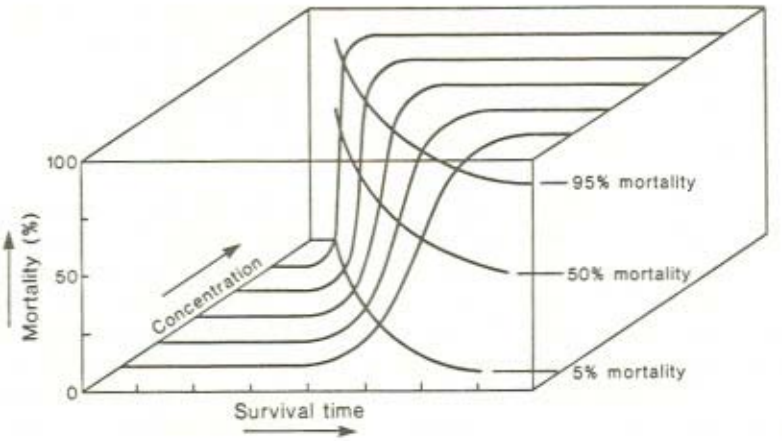


Figure 3 Survival time against concentration of DDT for rainbow trout Reproduced by permission of the Water Pollution Control Federation from Abram (1967)



After Skidmore (1974)

Figure 4 Interrelation between concentration, survival time and mortality Reproduced by permission of the British Veterinary Association from Skidmore, 1974

required for doubling of a population can range from decades for large mammals to hours for algae. There is an inherent stability of populations. Under natural conditions nearly all animal populations fluctuate irregularly between quite restricted limits. This comparative stability of populations is considered to be due to controlling factors which are density dependent (see, for example, Lack, 1954) and thus an effect at one point is compensated for at another. The ability of populations to withstand heavy mortality has been frequently demonstrated by the failure of most control programmes for pest species, for example, woodpigeon in Great Britain (Murton, 1965).

The forest spraying programme in eastern Canada for the control of spruce budworm (*Choristoneura fumiferana*) has been the largest and longest programme of chemical control of pests. From 1952 to 1981 seventeen million kilograms of pesticides were used. In 1975, 1.9 million hectares were sprayed in New Brunswick and the mortality of one species of songbird, the ruby-crowned kinglet (*Regulus calendula*) was estimated to be close to four hundred thousand. This was due largely to the use of an insecticide, phosphamidon, highly toxic to birds and thus the mortality was not typical of the entire operation over the years. Nevertheless, it was concluded (Pearce and Peakall, 1977) that 'after a quarter of a century of spray operations, both the ruby-crowned kinglet and the spruce budworm are alive and well in New Brunswick'. The best documented case of damage caused by forest spray programmes has been on Atlantic salmon (*Salmo salar*) (Elson, 1967;

Keenleyside, 1967). DDT at 1/2 lb/acre caused a 90–98 % reduction of underyearlings, 70 % reduction of small parr and 50 % reduction of large parr. Salmon numbers were reduced, but drastic effects on populations were mitigated by the fact that the reduction of young in any one year was spread over several years of returning adults. However, spraying in successive years would break down this resilience.

Canton and Slooff (1978) have compiled acute and chronic toxicity data for a variety of chemical control tests against a variety of aquatic organisms. The distribution of the ratio LC_{50}/chronic no-effect level for 77 cases was as follows: fewer than ten, 37.7 %; ten to one hundred, 41.5 %; one hundred to a thousand, 15.6 %; over a thousand, 5.2 %. The worst cases listed are sodium bromide for *Daphnia magna* (1050), toxaphene for *Chironomus plumosus* (1800), lead for *Salmo gairdneri* (3930), and guthion for *Pimephales promelas* (5760). None of these four cases are recorded in the open literature so that detailed evaluation has not been made. Using the safety margin of one hundred, some 20 % of these compounds would exceed this limit. The list of compounds is atypical for chemicals generally as studies are not normally carried out unless some problem is expected.

3.2 Extrapolation of Acute Toxicity to Behavioural Changes

The advantages of using behavioural changes to study the effects of chemicals was put forward by Warner *et al.* (1966) at the N.A.T.O. symposium 'Pesticides in the environment and their effect on wildlife' (Moore, 1966).

The following advantages were listed:

(1) Behaviour of the organism represents the final integrated result of a diversity of biochemical and physiological processes. Thus, a single behavioural change is generally more comprehensive than a physiological or biochemical change.
(2) Behavioural patterns are known to be highly sensitive to changes in the steady state of an organism. This sensitivity is one of the key values for its use in exploring sublethal toxicity.
(3) Behavioural measurements can usually be made without direct physical harm to the organism.

While the first criterion is reasonable, it is hard to evaluate. The advantages of the third criterion are more apparent than real. On the surface, it is attractive to have a non-destructive test, but at the end animals exposed to a chemical cannot be used in another experiment and are thus sacrificed.

The sensitivity of behavioural changes as an index of environmental effects can be more readily evaluated. Warner *et al.* (1966) validated their approach using the effect of toxaphene on goldfish (*Carassius auratus*). They found changes in several behavioural responses at 1.8 μg/l and a lesser degree of change at 0.44 μg/l. These values may be compared with a 96-hour median tolerance limit of 11 μg/l, giving a

sensitivity factor of 6–25. Ishio (1965) determined the ratio between the concentration required for 50% avoidance frequency and the lowest lethal concentration in fish and found that the ratios were small (0.46–2.23) for nine chemicals that he studied.

Kreitzer and Heinz (1974) investigated the effect of several chemicals on the avoidance response of young quail. The results, compared with LC_{50} values, are given in Table 3. Comparison of a rather modest level of avoidance (10–15%) to 5-day toxicity values gives ratios of 10–20. Correction for the length of the behavioural study as compared with the toxicity test would reduce this ratio. Higher ratios were obtained by Kreitzer (1980) studying the effect of toxaphene and endrin on the discrimination ability of bobwhite quail, but the lack of dose dependence makes these studies difficult to interpret.

Table 3 Comparison of dosage of chemicals causing 10–15% decrease of avoidance response to 5-day LC_{50} in Coturnix Quail chicks

Chemical	Dosage, 8 days ppm	LC_{50}, 5-day ppm	Ratio
Arochlor 1254	200	2900	14.5
Ceresan M	5	100	20.0
Chlordane	25	350	14.0
Dieldrin	5	57	11.5
Endrin	2	18	9.0

DDE at 50 ppm (LC_{50} 1350 mg/kg) had no significant effect. After Kreitzer and Heinz, 1974; Heath *et al.*, 1972.

Apart from sensitivity, there is the question of importance of behavioural alterations to the survival of the individual. It is hard to evaluate the significance of alterations in discriminatory ability. The effects of avoidance response are more obvious but still difficult to qualify. Decrease of nest attentiveness due to the presence of toxic chemicals has been demonstrated for the herring gull (*Larus argentatus*) in the Great Lakes (Fox *et al.*, 1978). Some studies have been made of the effect of such alterations on reproductive success. In the dunlin (*Calidris alpina*), a reduction of less than 10% in attentiveness reduced hatching success by more than 20% (Norton, 1972). A reduction in attentiveness resulting in a 1.2°C decrease in mean egg temperature of ring doves (*Streptopelia risoria*) was responsible for a 33% decrease of hatching success (Peakall and Peakall, 1973).

While behavioural alterations are a valuable tool for examining the effects of chemicals, the sensitivity of such methods does not appear to be greater than biochemical or physiological changes. The significance of behavioural alterations may be easier to assess in terms of impact on the individual than are biochemical or physiological changes but they are often more difficult to measure and quantify.

3.3 Extrapolation of Acute Toxicity to Physiological Changes

Biesinger and Christensen (1972) have compared for 19 metals the acutely toxic concentrations for *Daphnia magna* to concentrations required to cause 16% or 50% reproductive impairment. The ratios for all but one metal (tin, ratio 28) were less than ten for 50% impairment and for 16% impairment four exceeded ten and one (tin again) exceeded one hundred. Thus, despite the wide range of concentrations for acute toxicity (5–1500000 μg/l), the ratios of concentrations for acute and chronic toxicity are quite modest.

The induction of mixed function oxidase (MFO) enzymes is a sensitive indicator of the presence of pollutants. This system is quite different from most physiological mechanisms in that the MFO function has to be induced by xenobiotics whereas most other functions have feedback mechanisms to maintain homoeostasis. It is, therefore, not surprising that the ratio of acutely toxic concentrations to concentrations causing a physiological effect are rather larger for this sytem. For example, 2 ppm of dieldrin or 10 ppm of DDT induce the MFO activity causing a 3–4 fold increase in the rate of hydroxylation of steroid metabolism in pigeons (Peakall, 1967) which can be compared with 5-day LC_{50} values of 37 and 311 ppm, respectively (Heath *et al.*, 1972). The MFO system has been proposed as a monitor for petroleum hydrocarbons in fish (Payne and Fancey, 1982) and for dioxins in gull livers (Ellenton *et al.*, personal communication).

3.4 Extrapolation of Acute Toxicity to Histopathological Effects

Tucker and Leitzke (1979) stated that their conclusion and that of most other reviewers was that histopathological effects of pesticides on fish and wildlife are generally non-specific. More specifically, they concluded that pathology is a poor aid in the determination of biochemical mode of action and in the determination of the cause of kills in the field, and that pathological findings are not as good as time–toxicity curves in predicting chronic effects.

4 LABORATORY-TO-FIELD EXTRAPOLATION

4.1 Sensitivity of Laboratory Strains and Wild Strains

Any comprehensive discussion of laboratory-to-field extrapolation would exceed the scope of this review. Nevertheless, a few key principles can be introduced. First, in order to extrapolate from laboratory data to field effects one must take into account many considerations which can be formulated as follows:

(1) the degree to which the innate sensitivity to chemicals of wild strains may differ from tested laboratory stock;

(2) environmental variables which may alter the ultimate effect on the population;
(3) exposure variables which may alter the contact with and uptake of the chemical.

McEwen *et al.* (1973) tested the sensitivity of pen-reared ring-necked pheasants (*Phasianus colchicus*) against wild-trapped strains. Laboratory and field oral LD_{50} determinations were made by the same methods except that in the field experiment wild-trapped birds were fitted with microtransmitters and released to their habitat following oral dosing. Results showed that there was no significant difference in LD_{50} between the laboratory and field tests.

Wild-trapped *Daphnia magna* from the Tule Lake National Wildlife Refuge in California in 1964 produced 26-hour LC_{50} values nearly identical to those determined with a strain of *D. magna* removed from natural waters since 1915 (Tucker and Crosby, personal communication). Though there have been instances in which native wild strains of fish or invertebrates have become resistant to particular pesticides, most of the differences seen between laboratory and field assays can be attributed to environmental variables which alter the contact with and uptake of the chemical.

4.2 Extrapolation from Constant Concentrations to Fluctuating Concentrations

In most routine aquatic tests the concentration of the chemical is held constant. In the environment, the concentration may fluctuate if there is a pulse source of pollution or there may be a rapid decrease of concentration after a single discharge.

Abram (1967) studied the survival of harlequin fish (*Razbora heteromorpha*) in fluctuating concentrations of pesticides. In his experiment the fish were exposed alternately to a given concentration of the pesticide and to clean water for 5-hour periods, rapid changes being avoided, replacement being carried out on a constant flow basis. Abram found that the LC_{50} (7-day) was 1.5 ppm for constant concentration of an organophosphate compared to 4 ppm for fluctuating concentrations. The corresponding figures for DDT were 0.006 and 0.012 ppm. Abram considered that toxicity under conditions of discontinuous exposure tended toward that expected from the mean concentration unless there was rapid mortality at the highest concentration. This conclusion is supported by studies on effluents (Alabaster and Abram, 1965).

Experimental evaluation of decreases in concentration that would occur, often rapidly, after a single discharge does not appear to have been examined in aquatic toxicology. However, the mathematical basis for such studies exists (Butler, 1978).

One can conclude that, while wide fluctuations of toxicant concentrations can

be expected both with respect to time and space, the ability to model these changes exists.

4.3 Extrapolation to Other Physical Variables

A question that the evaluator of the impact of toxic chemicals faces is how the

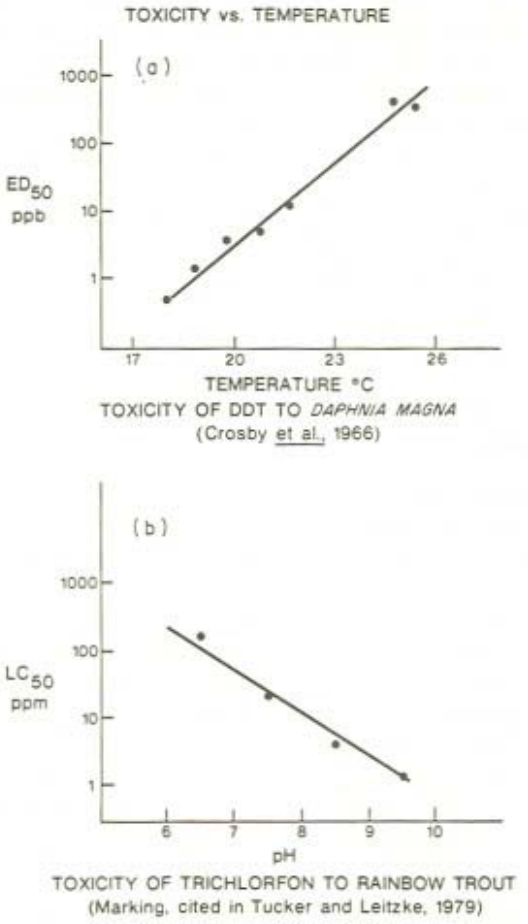


Figure 5 (a) Variation of toxicity of DDT to *Daphnia* with temperature. Reproduced by permission of Pergamon Press Ltd from Crosby *et al.* (1966) (b) Variation of toxicity of Trichlorfon to rainbow trout with pH. Reproduced by permission of the Water Pollution Control Federation from Abram (1967)

data generated at one temperature, or with specific physiochemical characteristics of water, apply to a variety of environmental conditions.

An obvious variable is temperature. The effect of toxicity of DDT to *Daphnia magna* (Figure 5a) was found to be highly sensitive to temperature (Crosby *et al.*, 1966). In this case, there was a 500-fold increase in toxicity for a six-degree rise in temperature. While DDT had one of the most marked temperature dependences, most of the 11 compounds tested had large temperature coefficients, although one was negative. These workers concluded that discrepancies in existing toxicity figures for *Daphnia* may be due primarily to temperature effects. Similar findings have been reported by many workers for fish (Tucker and Leitzke, 1979). In warm-blooded animals the problem of temperature can largely be ignored, although cold stress may somewhat increase the toxicity.

The pH of water can affect the toxicity of a chemical and a clear-cut example is plotted in Figure 5b. Other examples are cited in Tucker and Leitzke (1979). Knowledge of the physical chemistry (pK_A; rates of hydrolysis at varying pH) of the compound may allow reasonable prediction of toxicity changes. The effect of water hardness has been examined for the toxicity of several herbicides to fish (Inglis and Davis, 1972) and found to be negligible.

4.4 Differences in Exposure Variables between the Laboratory and the Field

Physicochemical variables which can extensively alter the outcome of field exposure are too numerous to be documented here. However, there are some very interesting instances worth special note. The Tule Lake *Daphnia*, mentioned before, were found in a healthy state in water which contained over 1.0 mg/l in suspended and dissolved DDT. This contrasted vividly with the laboratory-derived LC_{50} of only 4.4 μg/l (Crosby *et al.*, 1966). The reason for this gross discrepancy was that all but 0.1 μg/l of the lake DDT was bound to suspended organic particulates greater than 15 microns in diameter. *Daphnia* are unable to ingest such large particles.

Availability of the chemical accounts for most differences observed between laboratory and field effects. Factors such as temperature, pH and water hardness, can accelerate or impede chemical losses. Partitioning of the chemical may increase or decrease its availability. Results obtained with constant concentration exposures used in the laboratory contrast with rapidly changing concentrations often seen in the field. Nevertheless, there is a surprising degree of predictability between laboratory and field effects when the many factors are taken into account. Kills of wildlife by granular pesticides (e.g., kills of water fowl by granular carbofuran in British Columbia, NRCC, 1979) and seed-dressings (dieldrin coated seeds in the United Kingdom, Turtle, 1966) have been predicted from the basic toxicity data of these materials. Several years after the decision to ban DDT, the US Environmental Protection Agency published a review of the scientific aspects of the decision. The report gave new data showing

that populations began to recover as residues started to subside (EPA, 1975), thus confirming the correctness of earlier risk assessments in one of the most complicated field extrapolations of our time.

5 OVERALL COMPARISON OF LABORATORY AND FIELD EXPERIMENTS

5.1 Techniques for Toxicological Field Studies

One of the simplest approaches to converting laboratory studies into field studies is the use of caged animals put into the area to be examined. Caged fish were used in the studies of the effects of DDT on salmon (Kerswill and Edwards, 1967). This technique enables us to assess whether field conditions cause acute mortality to the test organism, but do not give indications of ecosystems toxicity. By increasing the size of the cage it is possible to study ecosystem effects. Flexible plastic columns have been used to enclose aquatic systems with several trophic levels and are capable of sustaining the population of the highest trophic level under study for at least one life cycle. Enclosures with a volume of 1700 m^3 and a depth of 29 m have been designed (Menzel and Case, 1977). These systems can be used to study pre-existing problems in the aquatic system or to add known quantities of toxicants. Even with a large system there are difficulties because the flow of nutrients is not the same as in the natural system and the effects of large predators are also missing.

Field trials are a widely accepted technique for testing pesticides. There are severe limitations to these studies. For conspicuous, comparatively easily censused organisms, such as birds, mortality can be masked by replacement since plots are small compared with the mobility of birds, food-chain effects can be masked by feeding outside the study area and reproduction and other critical effects are rarely studied. For other segments of the ecosystem such as non-target insects, there are other problems. Censusing is difficult where there are wide fluctuations of 'normal' population levels. For pesticides the expected exposure level and mode of exposure of the environment are known. This is not the case for toxic chemicals in general, nor is it feasible or desirable to contaminate wide areas for field trials except under exceptional circumstances.

Some techniques have been devised to limit the amount of the toxicant entering the environment. Enderson and Berger (1970) studied the effect of dieldrin on prairie falcons (*Falco mexicanus*) by tethering dieldrin-loaded starlings within their territory. The technique was successful in delivering the toxicant to the target but the results were confounded by the presence of high levels of DDE in the falcons. This finding points out one of the difficulties of field experiments, that the organism may be sufficiently contaminated with other toxicants to make the assessment of the results difficult or impossible. Surgical implantation of PCBs into wild puffins (*Fratercula arctica*) was successfully

carried out by Harris and Osborn (1981) and the desired tissue levels were achieved.

5.2 Environmental Variables which Affect the Population Outcome

At the Kern Research Area of the US Fish and Wildlife Service, records show several instances where ostracod and copepod populations rapidly exploded to fill the void caused by kills of daphnid populations in alkaline marshes. Within days the daphnid populations also recovered following use of short-lived insecticides. In these instances the temporary loss of an important link in the fish food chain (*Daphnia*) was ameliorated by expansion of alternative fish food organisms.

On the other hand, losses of slow-to-reproduce species such as eagles or salmon may be made up slowly, if at all. What is implied here is that, though the initial effect on a population may be similar to that experienced in the laboratory, the significance of the effect over time is tied to the potential and rapidity of repopulation of the particular species which was affected in its natural setting. In order to understand and predict field effects on populations we must have knowledge of natural natality and mortality rates. Henny (1972) provided such data for many common avian species; however, life tables are lacking for most wild vertebrates.

5.3 Extrapolation from Physiological Changes to Population Changes

The extrapolation from a demonstrated physiological change under laboratory or even controlled field conditions to a meaningful assessment of the impact on a population is difficult. Literature is full of comments like 'our data do indicate that small amounts of . . . could reduce . . . capacity for long-term survival' although few cases have been documented. This is more than likely due to the very small number of professional field investigators. The best documented case of field effects is the effect of egg shell thinning on the population of the peregrine falcon (*Falco peregrinus*). Cade's (1968) summary is worth bearing in mind:

> 'This remarkable record of resilience by a falcon population to direct human depredation stands in such marked contrast to the current, widespread loss in numbers of breeding Peregrine falcons as to emphasize once again the truly insidious nature of the agent responsible for the Peregrine falcon's decline. Down through the centuries, not all the falcon trappers, egg collectors, war ministries concerned for their messenger pigeons or misguided gunmen have been able to effect a significant reduction in the numbers of breeding falcons. But the simple laboratory trick of adding a few chlorine molecules to a hydrocarbon and the massive application of this unnatural class of chemicals to the environment can do what none of these other grosser, seemingly more harmful agents could do'.

The chronology of the egg shell thinning story is outlined in Table 4. The first observation, which in retrospect was related, was that of increases in the incidence of broken eggs in eyries by the early 1950s. The major finding that focused attention on the problem was the unprecedented population declines. The pattern of the exploration of the problem shows that the findings in one area, in this case the British Isles, was rapidly expanded to other areas. Experimental studies followed which related the cause to the effect and pinpointed the mechanism (not examined in detail here; the reference given is to illustrate the investigations into the subject) and to relate field investigations to laboratory studies.

Comparative studies (Table 5) have indicated that this problem would not have been predicted by studies on the commonly used laboratory species, with the possible exception of the mallard (Table 6), even if measurements of eggshell thinning had been carried out at the time that DDT was being tested.

Another major environmental problem caused by toxic chemicals was the reproductive failure of fish-eating birds in the Great Lakes of North America during the early 1970s. The data accumulated in Table 6 is an attempt, admittedly based on hindsight, to look at the available experimental data and a real world environmental problem and see to what extent we might have been able to predict the problem.

It appeared that both behavioural (or extrinsic) effects and direct embryonic effects (intrinsic) were involved in the failure of eggs to hatch. Based on experimental studies, neither dieldrin nor mirex would appear to have been present in sufficient quantity to cause an effect unless some marked species-to-species variation was involved. PCBs alone caused behavioural abnormality sufficient to explain the marked extrinsic effect but not the marked intrinsic effect. Two points of difference in experimental design between the study which demonstrated the marked extrinsic effect of PCBs on ring doves (Peakall and Peakall, 1973) and standard laboratory screening tests on avian reproduction should be mentioned. The first is that the marked reproductive failure was not noted until the second generation and that both normal and artificial incubation were used.

The separation of extrinsic and intrinsic effects on the reproduction of gulls was carried out by an egg exchange experiment (Peakall *et al.*, 1980). Under laboratory circumstances this can be achieved by a comparison of artificial and natural incubation.

The intrinsic factor does not seem to be accounted for in the laboratory studies of individual chemicals (McLaughlin *et al.*, 1963; Dunachie and Fletcher, 1969) and injection of mixtures of then known chemicals into eggs (Gilman *et al.*, 1978) support this conclusion. There are several other chemicals now known to be present in the eggs of Great Lakes herring gulls; of these the most likely candidate is the highly toxic tetrachlorodioxin but no good experimental data on the embryotoxicity of this compound exist.

Table 4 Chronology of events in eggshell thinning in peregrines

	1949–56	1961–62	1964	1967	1968	1969	1974	1975	1976	1977–80
Ecosystems										
Population		Disappearance of species over considerable portions UK (2) Holartic (3)								Population recovery linked to decreasing pesticide levels (9)
Intact Organism						Experimental linkage to DDT (3)		Correlation of laboratory to field studies (7)		
Organs	Broken eggs in nests (1)			Thinned eggshells UK (4), USA (5)			DDE levels in museum eggshells linked to DDE (6)			
Cells, tissues									Eggshell thinning linked to enzyme(8) changes in oviduct	
Molecular										

References: (1) Ratcliffe (1958), (2) Ratcliffe (1963), (3) Hickey (1969), (4) Ratcliffe (1967), (5) Hickey and Anderson (1968), (6) Peakall (1974), (7) Lincer (1975), (8) Miller *et al.* (1976), (9) Ratcliffe (1980).

Table 5 Levels of DDE required to cause significant eggshell thinning in a variety of avain species

	DDE levels causing 20% thinning in egg ppm wet weight	dietary ppm	Reference
Procellariiformes			
Leach's storm petrel (*Oceanodroma leucorhoa*)	12	—	Pearce *et al.* (1979)
Pelecaniformes			
Brown pelican (*Pelecanus occidentalis*)	8	—	Blus *et al.* (1972)
Double-crested cormorant (*Phalacrocorax auritus*)	10	—	Pearce *et al.* (1979)
Ciconiiformes			
Grey heron (*Ardea cinerea*)	25	—	Cooke *et al.* (1976)
Anseriformes			
Pekin duck (*Anas platyrhynchos*)	40	40	Peakall *et al.* (1973)
Black duck (*Anas rubripes*)	50–150	10–30	Longcore *et al.* (1971)
Falconiformes			
Peregrine (*Falco peregrinus*)	20	—	Peakall *et al.* (1975)
American kestrel (*Falco sparverius*)	—	6	Peakall *et al.* (1973)
American kestrel (*Falco sparverius*)	25	—	Lincer (1975)
Galliformes			
Chicken (*Gallus gallus*)	330 ppm causes no thinning		Cecil *et al.* (1972)
Japanese quail (*Coturnix coturnix*)	100 ppm causes only 5% thinning		Bitman *et al.* (1969)
Charadriiformes			
Herring gull (*Larus argentatus*)	160	—	Teeple (1977)
Atlantic puffin (*Fratercula arctica*)	30	—	Pearce *et al.* (1979)

Table 6 Comparison of the effects of chemicals on hering gulls in the Great Lakes with laboratory studies on other avian species

Type of experiment	Species	Named chemical	Pollutant Level: Dietary (ppm)	Pollutant Level: Tissue (ppm, lipid)	Observations	References
Field	Herring gull	PCB	—	1700	Increased embryonic mortality.	Gilman *et al.* (1977)
		DDE	—	280	Decreased nest defence	Fox *et al.* (1978)
		Mirex	—	62	and nest attentiveness.	Peakall *et al.* (1980)
		Dieldrin	—	5	Egg exchange experiments confirm	
		TCDD	—	0.012	both intrinsic and extrinsic.	
Laboratory	Ring dove	PCB	10	740	Decreased nest attentiveness leading to decreased hatching success in second generation birds. No effect on artificial incubation (i.e., no intrinsic effect).	Peakall and Peakall (1973)
		DDE	10	450	Significant reduction of courtship behaviour.	Haegele and Hudson (1977)
			50	2500	Significant reduction of total activity.	
		DDE	40	1800*	Marked increase in time to re-nest; significant decrease in survival of young.	Haegele and Hudson (1973)
		DDE	10	450*	Slight increase in time to oviposition.	Richie and Peterle (1979)
			40	1800*	Significant increase in time to oviposition.	
		PCB	8	610	Some increase in time required for	McArthur *et al.* (1983)
		DDE	1.7	100	breeding cycle; no effect on hatching,	
		Mirex	0.3	30	some effect on fledging.	
		Photomirex	0.095	9		
		PCB	28	1600	Marked increase in time required for	
		DDE	4.6	230	breeding; significant decrease in	
		Mirex	0.90	58	time spent brooding and feeding.	
		Photomirex	0.32	20	No effect on hatching success, marked decrease in fledging success.	
Laboratory	Mallard	Mirex	1	30	No effects on reproduction (artificial incubation).	Hyde *et al.* (1973)
			100	2960	No effect on hatching, some decrease of chick survival.	
	Quail		5, 40, 80	120, 1000 and 1920	No effect on fertility or hatchability.	Davison *et al.* (1975)
	Mallard	Dieldrin	4	190	Increased chick mortality; no effect on	Winn (1973)
			10	450	embryonic toxic effects.	
			30	650	Increased embryonic and chick mortality.	

* Ratioed from Haegele and Hudson (1977)

6 CONCLUSION

The degree of extrapolation that one is prepared to make depends on the degree of uncertainty of risk estimation that one is prepared to accept. It is universally acknowledged that there is no absolute certainty in ecotoxicological testing. What follows from this acknowledgement is that a decision has to be made on the basis of incomplete information. Hazard assessment has a degree of imprecision that scientists dislike and that calls for tough political decisions which politicians dislike. Nevertheless, the subject has to be tackled since the refusal of scientists and politicians to handle the problem can only lead to major environmental problems.

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