Appraisal of Tests to Predict the Environmental Behaviour of Chemicals
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4.5 *Bioaccumulation in Terrestrial Food Chains*

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4.5.1 INTRODUCTION

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 pollutant contained by individual animals. Conversely, this is not a very meaningful question for unstable compounds, although it will be relevant for any stable break-down or metabolic products.

Three topics need to be considered before we can appraise the amounts of persistent pollutants found in terrestrial animals, and whether or how it is possible to predict this distribution: the nature of food chains, the occurrence of pollutants in terrestrial species, and experimental data.

4.5.2 THE NATURE OF FOOD CHAINS

The only significant route of entry for persistent pollutants into terrestrial animals will, in many circumstances, be with the food. Animals eat only a limited range of species, and Elton (1927) first used the term food chain to describe the sequence in which a plant may be eaten by a herbivorous animal, which is in turn eaten by a carnivore, and so on. In this scheme, species are allocated to feeding, or trophic, levels, and members of one trophic level feed on organisms in the next lower trophic level, whilst they are preyed upon by organisms in the next higher trophic level. This scheme is useful as a short-hand description for the feeding relationships between different species, but it is over-simple:

(1) There are at least two major types of food chains. Most attention is commonly focused on those in which successive predators are larger than their prey. These are the so-called grazing food chains, but equally important are the processes of decay after death—the decomposer or detritivore food chains—without which life would soon become impossible, if only because essential elements would no longer recycle. Successive organisms in these food chains tend not to increase in size, most species of detritivore range in size from small to very small, and they include many bacteria, fungi and protozoa. There are also other, more specialized, feeding relationships such as parasitism and symbiosis.
(2) The term food chain implies a certain constancy about feeding patterns. In fact an animal's food will vary both with season, because both nutritional needs and availability of prey vary, and with the animal's size and age, because larger animals tend to take larger prey. Moreover, some animals feed on species from two or more trophic levels. Because of these complications, the term food web is sometimes used instead of food chain.

In practice it is usually difficult to discover the precise details of an animal's diet. Even the situation of livestock grazing in a field is deceptively simple, for grazing animals are usually selective in the species of plant that they eat (Duffey and Watt, 1971). There are in principle four ways in which the composition of the diet can be assessed: direct observation, dissection of guts, inspection of excreted or ejected gut contents, and tests of likely food items either by labelling with radioisotopes or by the use of serological techniques.

One example of the nature of food webs must suffice. Southern (1954) studied the food of individual tawny owls (*Strix aluco*) on the Wytham estate, near Oxford, an area of about 1000 acres (445 ha), predominantly deciduous woodland with some parkland, young plantations, rough grassland and farmland, which normally contained about 20 breeding pairs of tawny owl. Tawny owls maintain separate territories, and eject the indigestible fragments of their prey in pellets, at definite, though changeable, positions within their territories. Southern collected all pellets from known pellet-stations every month for 8 years, from 1945–1952, and identified the contents.

Table 4.5.1 Numbers of different species of vertebrate prey taken by tawny owls (*Strix aluco*) in Wytham from 1945–1952. Quantities are also expressed as 'prey units', by using conversion factors based on the estimated mean weight of the prey taken of each species (data from Southern, 1954).

Species of pray	No. of individuals	Conversion factor	'Prey units
Wood mouse (*Apodemus sylvaticus*)	2783	1	2783
Bank vole (*Clethrionomys glareolus*)	2920	1	2920
Short-tailed vole (*Microtus agrestis*)	1269	1	1269
Common shrew (*Sorex araneus*)	1146	0.5	573
Pygmy shrew (*Sorex minutus*)	174	0.2	35
Water shrew (*Neomys fodiens*)	38	0.75	29
Mole (*Talpa europaea*)	436	5	2180
Rabbit (*Oryctolagus cuniculus*)	165	10	1650
Brown rat (*Rattus norvegicus*)	79	5	395
Water vole (*Arvicola amphibius*)	8	5	40
Weasel (*Mustela nivalis*)	4	5	20
Birds (14 species, predominantly finches, particularly the chaffinch, *Fringilla coelebs*)	486	1	486
Bats	4	0.25	1
	9494		12363

Southern found that owls took both vertebrate and invertebrate prey. It was difficult to calculate the actual quantities eaten of invertebrates, because their remains are not easily assigned to separate individuals, so we will consider the vertebrate prey first. A wide range of prey species were taken (Table 4.5.1), with considerable variations between seasons and years (Figure 4.5.1). There was a pronounced seasonal fluctuation in the relative importance of small and large mammals. Rodents comprised nearly 80% of the food eaten in the four months after leaf-fall, when the ground vegetation has died, compared to 30–45% in the summer and early autumn, when larger mammals predominated in the diet. Similar seasonal cycles occurred for the relative numbers of rabbits and moles, with maximum consumption of individuals from both these species related to the time at which these animals breed. There were also pronounced yearly variations in the relative numbers eaten of rodents and of larger mammals. Territories differed too in the proportions taken of different prey species, which appears to reflect not only differences between territories in the availability of prey, but also reflects the feeding preferences of individual owls. The principal invertebrate prey were beetles, especially cockchafers (*Melolontha melolantha*) taken in the

Table 4.4.2 Bioconcentration factors for selected compounds in relation to various parameters

Substance	BCF	Species and other parameters	Reference
γ-HCH	84	shrimp, *Penaeus duorarum*	Schimmel *et al.* (1977)
	180	fish, *Pimephales promelas*	Veith *et al.* (1979)
	140	mussel, *Mytilus edulis*	Ernst (1977)
	460	polychaete, *Nereis virens*	Goerke and Ernst (1980)
	1 240	polychaete, *Lanice conchilega*	Ernst (1979)
	490	fish, *Cyprinodon variegatus*	Schimmel *et al.* (1977)
Pentachlorophenol	296	fish, *Gambusia affinis* (model ecosystem)	Lu and Metcalf (1975)
	326	mussel, *Mytilus edulis* (5 °C)	Ernst (1979)
	304	mussel, *Mytilus edulis* (10 °C)	Ernst (1979)
	324	mussel, *Mytilus edulis* (15 °C)	Ernst (1979)
	770	fish, *Pimephales promelas*	Veith *et al.* (1979)
Endrin	1 479	fish, *Gambusia affinis*	Neely *et al.* (1974)
	1 900	mussel, *Mytilus edulis*	Ernst (1977)
	2 700	oyster, *Crassostrea virginica*	Mason and Rowe (1976)
Hexachlorobenzene	7 880	fish, *Salmo gairdneri*	Neely *et al.* (1974)
	1 166	fish, *Gambusia affinis*	Lu and Metcalf (1975)
	18 500	fish, *Pimephales promelas*	Veith *et al.* (1979)
Methoxychlor	8 300	fish, *Pimephales promelas*	Veith *et al.* (1979)
	1 545	fish, *Gambusia affinis* (ecosystem)	Metcalf (1972)
p,p'-DDT	100 000	fish, *Pimephales promelas* (266 days exposure)	Jarvinen *et al.* (1977)
	29 400	fish, *Pimephales promelas* (32 days exposure)	Veith *et al.* (1979)
PCB, Aroclor 1248	70 500	fish, *Pimephales promelas*	Veith *et al.* (1979)
	60 000	fish, *Pimephales promelas* (male)	Defoe *et al.* (1978)
	120 000	fish, *Pimephales promelas* (female)	Defoe *et al.* (1978)
PCB, Aroclor 1254	10 965	fish, *Pimephales promelas* (5 °C)	Veith *et al.* (1979)
	75 858	fish, *Pimephales promelas* (25 °C)	Veith *et al.* (1979)
	5 888	fish, *Lepomis cyanellus* (5 °C)	Veith *et al.* (1979)
	69 183	fish, *Lepomis cyanellus* (25 °C)	Veith *et al.* (1979)
	7 413	fish, *Salmo gairdneri* (5 °C)	Veith *et al.* (1979)
	14 125	fish, *Salmo gairdneri* (20 °C)	Veith *et al.* (1979)
	85 000–101 000	oyster, *Crassostrea virginica*	Lowe *et al.* (1972)
PCB, Aroclor 1260	194 000	fish, *Pimephales promelas*	Veith *et al.* (1979)
	160 000	fish, *Pimephales promelas* (male)	Defoe *et al.* (1978)
	270 000	fish, *Pimephales promelas* (female)	Defoe *et al.* (1978)

summer, and earthworms, principally on open ground, where numbers eaten tended to increase after rainfall. Rainfall had less effect on the consumption of earthworms from more shaded areas. Less detailed data from France and Germany suggested that the diet of tawny owls probably differs from one region to another.

The important conclusions for our purposes are that, for this species at least:

(1) Different individual owls can experience very different exposures to pollutant in their food, because their food is so diverse.
(2) Seasonal changes in diet imply that exposures of particular individuals will vary with season.

4.5.3 THE OCCURRENCE OF POLLUTANTS IN TERRESTRIAL SPECIES

An enormous amount of data has been published on the amounts of pollutant found in terrestrial organisms, and at least one entire journal, the *Pesticides Monitoring Journal*, contains nothing but analytical results, and their interpretation, for pesticides, with a large proportion referring to terrestrial species. Only a small proportion is of direct relevance for assessing the magnitude of bioaccumulation, because amounts of pollutant in the food of specimens that are analysed are usually unknown. For heavy metals, several authors have commented on the paucity of relevant data (Hughes *et al.*, 1980).

Amounts of pollutants in species from different trophic levels are commonly compared by concentration factors, defined as the concentration of pollutant in one species divided by the concentration of the pollutant in the food of that species. Interpretation of such factors is complicated by many variables, which include:

(1) The possibility that concentrations of residues are not directly proportional to the magnitude of the exposure, when the concentration factor will vary with the degree of exposure.
(2) Difficulties of establishing what is eaten. Invariably assumptions have to be made about the nature of the diet before concentration factors can be calculated (e.g. Williamson, 1979). In some instances not all of a prey's tissues are eaten, when whole-body concentration in prey may give an inaccurate estimate of the predator's dose.
(3) Lack of information on the frequency-distribution of concentrations found in different individuals. Use of a single concentration factor for pollutant levels in a species and its food frequently implies that both food and consumer are sampled randomly, with individual

concentrations conforming to unimodal distributions. In practice, both these assumptions may be false.

Carcasses of predatory birds received at Monks Wood during the years 1963–1977 were allocated to one of three categories: death by trauma, disease/starvation and other, unknown, causes (Cooke *et al*., 1979). Concentrations of both DDE and HEOD in the livers were significantly lower in birds dead from trauma than in birds dead from unknown causes (Table 4.5.2).

Analysis for PCBs gave a similar pattern, except for the kestrel, which showed no significant difference between these two categories of death. Whatever the causes of these differences, one relevant deduction is that great care would be needed to get a realistic picture of the amounts of pollutants found in the livers of these four species of bird.

More positive methods of sampling may also produce biases. I have discussed earlier (Moriarty, 1978) how the amount of pollutant in one tissue or organ varies with duration of exposure. There is a considerable amount of evidence to show that age and/or size, time of year, and details of the physical environment can also affect concentrations of pollutants in biota.

(4) Concentration factors should be derived from comparable analytical results. Not infrequently concentrations for specific tissues or organs in a predator are compared with concentrations for whole bodies of their presumed prey. This is likely to give misleading results, and it is a nice question which tissues of, say, an earthworm are strictly comparable with which tissues of, say, the bird that eats the earthworm. Mean concentrations in whole bodies have a semblance of greater comparative value, although the precise numerical value of a concentration factor will depend on the way concentrations are expressed: in particular, as fresh, dry, lipid or protein weight of organism. No one system is completely satisfactory.

Mass of pollutant is the primary unit for studying transfer along food chains, and concentrations are used primarily to compensate for the different weights of different organisms. Concentrations should therefore be based on fresh weights unless there are additional special considerations. Much of the published literature bases concentrations on dry weights of organisms, which will give values roughly four times as high as concentrations based on fresh weights.

There are good reasons why other units of concentration are sometimes to be preferred, but they are irrelevant for the assessment of transfer of pollutant along food chains. Thus, concentrations of organochlorine insecticide in fatty tissues may indicate the degree of risk to that animal at times of starvation (Jefferies and Davis, 1968) or hibernation (Jefferies, 1972), and the concentration of organochlorine

Table 4.5.2 Effect of cause of death on the frequency distribution (%) of residues of three pollutants in livers from four species of bird (data derived from Cooke *et al.*, 1979)

Species	Cause of death	HEOD (p.p.m.) 0–0.9	HEOD 1–9.9	HEOD >9.9	HEOD No. of analyses	DDE (p.p.m.) 0–0.9	DDE 1–9.9	DDE 10–99	DDE >99	DDE No. of analyses	PCBs (p.p.m.) 0–0.9	PCBs 1–9.9	PCBs 10–99	PCBs >99	PCBs No. of analyses
Heron	Trauma	73	22	5	60	35	38	23	3	60	25	48	21	6	52
(*Ardea cinerea*)	Unknown	32	43	25	69	12	32	48	9	69	8	24	65	4	51
Sparrowhawk	Trauma	72	27	1	93	20	57	20	2	93	24	60	16	—	75
(*Accipiter nisus*)	Unknown	52	35	13	91	18	37	38	7	91	20	45	35	—	71
Kestrel	Trauma	65	30	5	101	50	38	12	—	101	42	47	11	—	72
(*Falco tinnunculus*)	Unknown	23	31	46	119	25	51	20	3	119	33	53	13	—	90
Barn owl	Trauma	69	28	3	123	70	28	2	—	123	66	32	2	—	97
(*Tyto alba*)	Unknown	30	40	30	93	35	38	24	3	93	38	43	18	—	65

insecticides in the brain, or the concentrations of some heavy metals in the kidney, may indicate the probability of death or of lesser malfunction (O'Brien, 1967; Task Group on Metal Accumulation, 1973). Even in these instances, it is still desirable to know the total mass of pollutant contained in individual organisms as well.

4.5.3.1 Earthworms

Earthworms are of particular interest. It is relatively easy to assess the degree of contamination of an earthworm's environment, and information about the relationship between field exposures to pollutants and the consequent levels of body residues is more extensive than for any other terrestrial group. Moreover, earthworms are prominent in the limited number of experimental studies on pollutants in food chains. It should be remembered though that earthworms are in intimate contact with the surrounding soil, so that food may not be the sole source of their residues. If a significant proportion comes by intake through the external body surface, the real concentration factor for pollutant ingested with the food will be less than is apparent.

We do not know what proportion of pollutants found in earthworms enters across the body surface. Earthworms can absorb lipophilic pesticides from water and soil through their body surface, although diffusion of pesticides through soil is so slow that earthworms kept in soils that are stirred periodically attained steady concentrations of dieldrin that were about 50% higher than for earthworms kept in unstirred soils (Lord *et al.*, 1980). Some of the species that penetrate deep into the soil form permanent burrows (Edwards and Lofty, 1972), when bodily contact with the soil may be less important than for species that do not form burrows.

Beyer and Gish (1980) applied dieldrin, heptachlor or DDT at 0.6, 2.2 or 9.0 kg active ingredient/ha (0.5, 2 or 8 lb/a) to individual 10 m square plots. Samples of earthworms and soil were analysed for the next 11 years. There were four species of earthworm (*Aporrectodea trapezoides, A. turgida, Allolobophora chlorotica* and *Lumbricus terrestris*), but any differences between species were ignored, and most specimens were taken from the top 20 cm of soil. Soil samples were taken from the top 3.5 cm.

In general it was practicable to describe changes with time of residues, in both soil and earthworms, by linear regression (Table 4.5.3). The major exceptions were for heptachlor, which declined to trace levels in soils within 2–6 years, and for the breakdown products heptachlor epoxide and DDE in soil, where residues rose for some time after application. Earthworms contained higher concentrations of dieldrin, heptachlor epoxide and of DDE than the soil, but tended to have lower concentrations of p, p'-DDT. This last result indicates that any DDT absorbed by earthworms is metabolised rapidly to DDE. Heptachlor was not detected in earthworms. These results suggest

Table 4.5.3 Concentrations, and rates of loss, of organochlorine insecticides in soil and earthworms from experimental plots in Maryland, USA, for a period of 11 years, from application of pesticide in 1966, until 1977. Concentrations refer to dry weight of both soils and earthworms, and each value is the arithmetic mean for two replicates. v.p. indicates very persistent. Half-lives and initial residues were estimated by linear regressions. It should be noted that the half-life for earthworms measures how rapidly residues declined in the population of earthworms, which occurred concurrently with the decline in soil concentrations (data from Beyer and Gish, 1980)

Insecticide	Application rate (kg/ha)	Residues (p.p.m.) present initially after application		Half-life (years)	
		Soil	Earthworms	Soil	Earthworms
Dieldrin	0.6	0.46	7.8	2.6	1.7
	2.2	1.3	21	4.1	2.2
	9.0	4.0	54	12.5	4.5
p,p′-DDT	0.6	0.35	0.19	1.8	2.5
	2.2	1.1	0.20	2.7	3.2
	9.0	5.7	0.88	2.7	3.8
DDE (applied as DDT)	0.6		0.87	v.p.	3.1
	2.2		3.0	v.p.	4.9
	9.0		7.4	v.p.	v.p.
Heptachlor epoxide (applied as heptachlor)	0.6		1.8	3.6	2.3
	2.2		6.8	2.8	2.2
	9.0		17	3.3	5.6

that, for chemically stable organochlorine insecticides or conversion products, earthworms tend to have higher concentrations than the soil. Too much emphasis should not be placed on the actual figures: concentrations in earthworms would be about 25% of those shown in Table 4.5.3 if based on wet weights of earthworms. Moreover, earthworms were not entirely devoid of soil in their guts when analysed: Davis (1971) found in laboratory tests on *Lumbricus terrestris* that gut contents were about 11% of the total dry weight and contained 23–40% of the total content of dieldrin residues after 20 days' exposure to compost with 32 p.p.m. dieldrin. The half-lives for residues in soils do not always coincide with those for residues in earthworms: in particular levels of dieldrin decreased more rapidly in earthworms than in soils.

If one allows for the results being based on dry weights, one may conclude that there has been a modest increase in concentration from soil to earthworm.

Laboratory experiments with soils in containers suggested that many factors can influence such results (Davis, 1971). Results depend on the

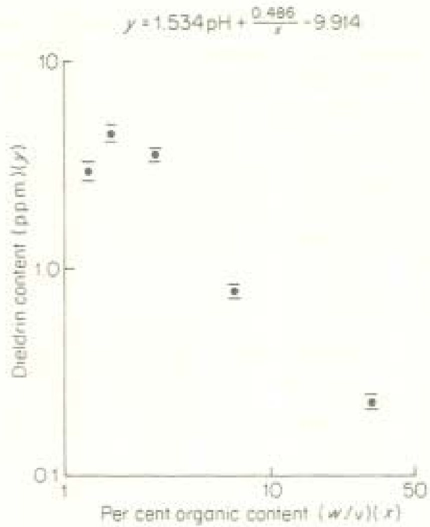

Figure 4.5.2 Graph of the dieldrin concentration (y, based on fresh weights) in earthworms (*Allolobophora caliginosa*) kept in one of five soils whose organic content (x) ranged from peaty loam to loamy sand. 95% of the total variance between soils was accounted for by the equation, which takes account of soil pH as well as organic content. Horizontal bars indicate standard errors. (from Davis, 1971)

species, which may interact with the mode of exposure, and/or the insecticide (Table 4.5.4). Soil properties also can affect residues. The concentration of dieldrin in *Allolobophora caliginosa* after 4 weeks' exposure varied 20-fold according to soil type. The effect of soil depended largely on its content of

Table 4.5.4 Residues of dieldrin and DDT residues in two species of earthworms after two types of exposure. Exposure continued for 4 weeks, concentrations based on fresh weights (data from Davis, 1971)

Exposure	Residue	Species: *Allolobophora caliginosa*	Species: *Lumbricus terrestris*
17 p.p.m. dieldrin in soil	Dieldrin	27.3±0.66	11.55±0.54
Fallen apple-leaves from all orchard sprayed with DDT	*pp*′-DDT	0.25	1.32
	op′-DDT	trace	0.19
	pp′-DDE	trace	0.20

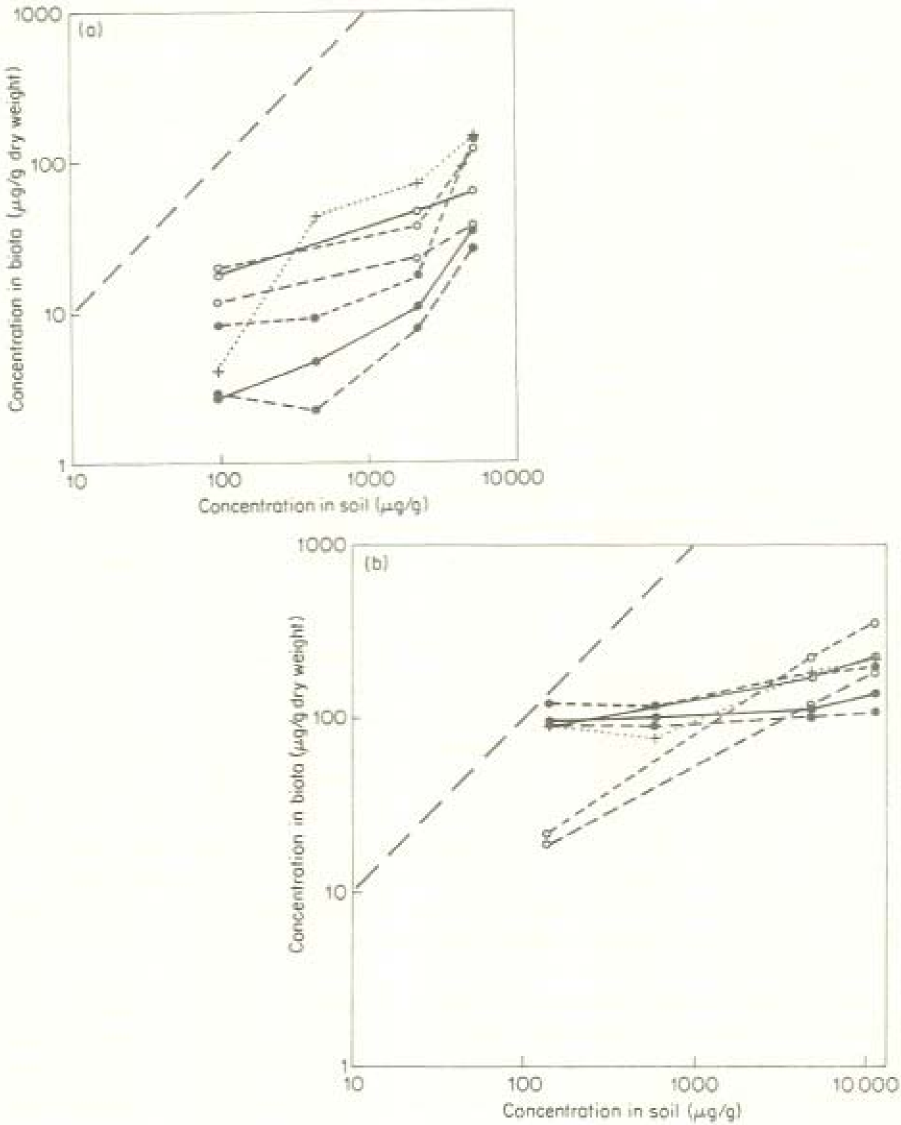

Figure 4.5.3 Concentrations of a (a) lead, (b) zinc, in the top layer of soil (0–5 cm deep), and in four animal species, along a transect from derelict mine workings near Wrexham, North Wales. Sites were adjacent to the mine, 100 and 500 m downward and 10 km away +···+ denotes earthworms (*Lumbricus terrestris*), — denotes the common shrew (*Sorex araneus*), – – – denotes the wood mouse (*Apodemus sylvaticus*), and ---- denotes the field vole (*Microtus agrestis*). ● indicates a mean body concentrations; ○ indicates concentrations in the diet: ground cover vegetation for *Microtus agrestis*, cover vegetation and endosperm for *Apodemus sylvaticus*, and invertebrates for *Sorex araneus*. The diagonal dashed line indicates concentrations that equal those in the soil (data from Roberts and Johnson, 1978)

organic matter (Figure 4.5.2), but soil pH was also significant, and between them these two factors accounted for 95% of the total variance in dieldrin content of the worms.

Wheatley and Hardman (1968) examined data from their own and other work and concluded that, for a range of earthworm species and organochlorine insecticides (aldrin, dieldrin, DDT and lindane), from several sites, that $C = aX^b$, where C is the concentration of insecticide in the earthworm (based on fresh weights), X is the concentration in soil, and $b = 0.79$. Concentration factors in earthworms become less than unity at soil concentrations above about 10 p.p.m., and with concentration factors of 5 to 10-fold for soil concentrations of 0.001–0.01 p.p.m. These are broad generalisations, affected by factors of the type already discussed.

The relationship between concentration of metals in earthworms and their surrounding soils varies with the metal (Hughes *et al.*, 1980). Several reports show that earthworms contain lower concentrations of lead than the soil. The sole exception, for concentrations based on dry weights, is for *Dendrobaena rubida* in soil heavily contaminated with mine waste: earthworms contained 4160 ± 930 p.p.m. of lead, whilst soil samples had only 1713 ± p.p.m., a concentration factor of 2.43 (Ireland, 1975). Factors in other studies ranged from approximately 1.0 to 0.04. Cadmium, by contrast, had concentration factors within the range 3.8–22.5. Body-levels of zinc, an element essential to life, are regulated by many species within relatively constant limits, when the concentration factor would be expected to decrease as the concentration of zinc in soil increases. Zinc concentration in *Lumbricus terrestris,* taken at various distances from an area contaminated by lead and zinc from an abandoned mine, changed by a factor of two-fold for a fifteen-fold change in soil concentrations (Roberts and Johnson, 1978) (Figure 4.5.3). At the same time, lead concentrations in earthworms were a constant proportion of those in the soil.

In summary, these data are sufficient to suggest that a universal statement cannot be made for earthworms about the increase in concentration, or lack of it, to earthworms from their environment. Whether or not concentrations increase depends on both the pollutant and a range of environmental variables.

4.5.3.2 Food Webs

For many terrestrial species, particularly vertebrates, food is likely to be the major or sole source of pollutants. There are extensive sets of data on pollutant levels in field specimens, but never is it certain how near pollutant levels are to a steady-state, and there is little or no information on the extent to which contamination of the food fluctuates—often there is no information of any sort on the degree of exposure. Direct calculation of concentration factors is therefore virtually impossible.

Possibly the first study on transfer of pesticides along food chains was made at Urbana, Illinois (Barker, 1958). 1400 elm trees (*Ulmus americana*) on the 400-acre university campus were sprayed with technical grade DDT twice yearly, in June or early July, and again between mid-July and mid-August, each year from 1949–1953, at a rate of 1–1.5 lb/tree. Some time after the first year's applications, but before any sprays in 1950, considerable numbers of dying American robins (*Turdus migratorius*) were reported, particularly after rain. These birds exhibited the typical symptoms of tremor that are associated with DDT poisoning, and brains were analysed, by the Schechter–Haller method, for DDT and DDE. One cannot put too much reliance on results from this analytical method: quantities are estimated by intensity of colour in solution, and potentially many compounds can interfere, DDD in particular (Miskus, 1964). However, it is reasonable to deduce from the results (Table 4.5.5) that:

(1) There was a bimodal distribution of residue concentrations.
(2) Many of these birds were killed by DDT, with a modal concentration of about 60–70 μg DDT/g of brain.
(3) Birds with less than 10 μg/g probably died from other causes. Two of the six individuals contained no detectable residues, nor did two healthy robins from an uncontaminated area that were shot. This illustrates the point that care is needed to take unbiased samples if one wishes to estimate the magnitude of the residues that occur in a population. This sample was knowingly biased in favour of birds suspected of having high residues.

Barker considered the likely pathways by which robins had acquired these residues. Most of the DDT in the soil occurred in the top layer:

Depth of soil sample	*Concentration after the first spray application, 1950.*
0–¼ inch	17.8 μg DDT/g soil
¼–2 inch	5.9–9.5 μg DDT/g soil
2–3 inch	0.9 μg DDT/g soil

Table 4.5.5 Concentrations (based on fresh weights) of DDT in the brain of adult robins (*Turdis migratorius*) found dead or dying on or near the university campus, Urbana, Illinois, from May 1950–May 1952 (data from Barker, 1958)

Range of concentrations (μg/g)										
0–10	11–20	21–30	31–40	41–50	51–60	61–70	71–80	81–90	91–100	>100
No. of birds										
6	1	3	1	3	5	7	3	1	1	1

Leaves on the trees contained higher concentrations:

Time samples taken	*Concentrations (based on fresh weights)*
1 day before the second spray application, 1950	15, 30, 109 and 206 μg DDT/g leaf
1 day after the second spray application, 1950	174, 253 and 263 μg DDT/g leaf

When the leaves fell that autumn, three samples contained 20, 23 and 28 μg DDT/g leaf, which was similar to levels in the surface soil layer. Fallen leaves were chaffed and mulched by machines, and left on the lawns.

Earthworms of various species were also analysed, entire, and contained from 33–164 μg DDT/g body fresh weight.

Deaths of robins were reported particularly after heavy rains, when earthworms tend to come to the soil surface, and Barker suggested that earthworms concentrated DDT residues by selective feeding on sprayed leaf mulch, and that robins ingested their lethal dose of DDT with the earthworms they ate.

Barker's paper set the pattern, in one very important aspect, for nearly all subsequent work. Results were expressed as concentrations, and rarely is it possible to calculate, from the published data, the mass of pesticide. Moreover, for large organisms, particularly vertebrates, the total body burden cannot be determined, because only some specific tissues are analysed.

Moore and Walker (1964) analysed the breast muscle from 85 bird corpses collected in the field '. . . generally in the absence of circumstances suggestive of poisoning'. They also analysed 58 eggs, and both sets of analyses showed that concentrations of *p, p'*-DDE and dieldrin were highest in predators, and were less in omnivores and carnivores, a pattern which has since been found to be widespread (Moore, 1965; Stickel, 1973). These results indicated clearly that organochlorine insecticides and/or their more stable metabolites become widely dispersed and occur in many species. Reasons for the link between feeding habits and residue concentrations were uncertain. Differential contamination of food, or physiological differences between species, were suggested as possible causes, but a widespread view now is that successive predators will inevitably acquire higher residues than their prey contain. This view is not well-founded, and we will consider why in the next section.

4.5.4 EXPERIMENTAL DATA

4.5.4.1 Models

The experimental evidence on bioaccumulation is fragmentary and interpretation will be helped by a preliminary, simplified, description of the topic. If a predator neither excretes nor metabolises any of the prey's pollutant, then the concentration of pollutant in the predator will exceed that in the prey as soon as the predator has eaten more than its own body weight of that prey species. This does assume 100% assimilation of ingested pollutant, and we will return to this point later, but lower assimilation rates do not affect the principle of the argument. In practice no pollutant is retained completely, so whether or not the concentration factor exceeds unity depends on the balance between the rate of intake and the rate of loss by excretion and metabolism. A steady-state is established when these rates are equal.

Let us consider a prey species whose individuals have all attained the same steady-state concentration of pollutant, and let us suppose that the pollutant within an individual predator can be regarded as a single homogeneous compartment (Moriarty, 1975).

Then

$$Q_{00} = \frac{R}{k_{01}}$$

where Q_{00} (μg) is the steady-state mass of pollutant within the predator, R (μg/day) is the rate of intake of pollutant, and k_{01} (μg/day) is the rate constant for excretion plus metabolism.

$$R = fc$$

where f (g/day) is the weight of food consumed and c (μg/g) is the concentration of pollutant in the food.

Let W be the predator's weight in grams. Then

$$Q_{00} = \frac{fc}{k_{01}}$$

and the concentration factor (see p. 261) is

$$\frac{1}{W}\frac{f}{k_{01}} = \frac{w}{k_{01}}$$

In other words, pollutant concentration is higher in the predator than the prey if w, the rate of food consumption as a proportion of the predator's body weight, exceeds the rate constant for excretion plus metabolism.

In practice, many qualifications are needed for this simple, but revealing, conclusion. These include:

(1) The amount of pollutant within the predator decreases as the percentage assimilation decreases.

(2) Growth in the predator increases both food consumption and the mass of tissue within which the pollutant is distributed.

(3) The proportions of pollutant that are assimilated, excreted and metabolized may all alter with exposure rate.
(4) Different tissues and organs within an organism may nave different concentrations of pollutant, and approach a steady-state at different rates.

Within the assumptions of this model, since

$$t_{1/2} = \frac{0.693}{k_{01}}$$

where $t_{1/2}$ (days) = the half-life for assimilated pollutant, on the assumption that loss occurs at an exponential rate, if

$$\frac{w}{k_{01}} = 1$$

then

$$t_{1/2} = \frac{1}{1.443w}$$

in general, the value of w decreases as W increases (Kenaga, 1972), although it is not easy to get precise data for animals in the field. As an example, Brown (1976) found, for nine species of predatory birds that w ranged from 0.23 for a bird weighing 167 g to 0.06 for a bird weighing 3705 g (Figure 4.5.4). An

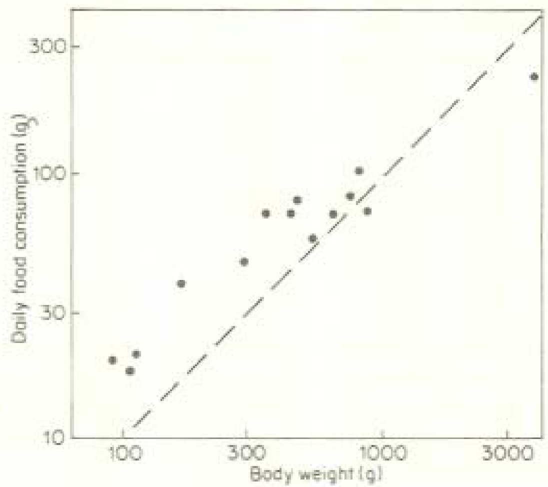

Figure 4.5.4 The effect of body weight on food consumption during the summer by individual predatory birds of nine species. – – – indicates a daily food consumption of 10% of the body weight (data from Brown, 1976)

Table 4.5.6 Half-lives for loss of pollutant from a predator, regarded as one compartment with exponential loss, that will ensure concentration factors of unity in steady-state conditions, for a range of feeding rates, and for two assimilation rates

Daily food intake as a proportion of body weight	0.01	0.05	0.10	0.25	0.50	1.00
Half-life (days) with 100% assimilation	69.30	13.86	6.93	2.77	1.39	0.69
Half-life (days) with 10% assimilation	693	138.6	69.3	27.7	13.9	6.9

illustrative series of half-lives, for 10 and 100% assimilation of ingested pollutant, are given in Table 4.5.6. These show, within the assumptions of the model, that larger predators will have lower concentrations than will small predators, simply because large animals tend to eat less, per unit of body weight, than do small animals. This then permits a range of possibilities, from the situation where concentration factors increase, at a declining rate, at successive trophic levels towards the top predators, via situations with shorter half-lives where intermediate trophic levels have the highest concentration factors, to situations where the concentration factor decreases at each successive trophic level (Table 4.5.7).

These calculations are solely heuristic, but do demonstrate that as one ascends a food chain the likelihood increases that the pollutant concentration will decrease in successive trophic levels. One may conclude that both assimilation rates and elimination rates must be known, or estimated before concentration factors can be predicted.

4.5.4.2 Feeding Trials

Few experimental studies have been made on the retention of pollutants by terrestrial species fed on other animal species. In one of the earliest studies, American woodcock (*Philohela minor*) were fed for up to 60 days on earthworms (*Lumbricus terrestris*) that had been exposed to heptachlor (Stickel *et al*., 1965). Worms were kept for 7 days in peat with 0.0705 g or 0.0044 g heptachlor/149.8 g (1 l) of medium (471 or 29.4 p.p.m. w/w), and chemical analyses suggested that the worms contained on average 2.86 or 0.65 p.p.m. respectively (based on dry weights) of heptachlor residues (heptachlor plus heptachlor epoxide). Twelve birds were fed on worms that contained an estimated 0.64 p.p.m.: one bird died after 45 days, two were killed after 60 days, and 7 others died or were killed during the next 11 days, when they were fed on a restricted quantity of worms. The mean body burden was 52.7 μg, with a range of 17–146 μg. There was a slight suggestion, not significant statistically, that those birds partially starved before they were

Table 4.5.7 The steady-state concentration for pollutant with different half-lives in five successive trophic levels, assuming a concentration of 1 μg/g in individuals of the first level. Animals are treated as single compartments, with first-order kinetics for intake and loss of pollutant, and 10% assimilation of the ingested pollutant

Trophic level	Daily food intake as a proportion of body weight	Steady-state concentrations for half-lives (days) of 10	50	150	Concentration factors for half-lives (days) of 10	50	150
1st	1.00	1	1	1	—	—	—
2nd	0.04	0.58	2.9	8.7	0.58	2.9	8.7
3rd	0.20	0.17	4.2	37.5	0.29	1.4	4.3
4th	0.10	0.024	3.0	81.1	0.14	0.72	2.2
5th	0.05	0.0017	1.1	87.8	0.07	0.36	1.1

analysed had higher burdens than the other three birds (mean logarithms of weight (μg) ± standard errors: 1.72 ± 0.17 and 1.45 ± 0.15 respectively). The mean body content of heptachlor epoxide, adjusted where appropriate to compensate for loss in body weight due to starvation, was 1.7 p.p.m. (based on dry weights), nearly three times the concentration in the worms.

Of 12 birds on the higher dosage, fed on worms that contained an estimated 2.86 p.p.m. of heptachlor residues, 10 had died by the 52nd day. One of the two survivors was killed the next day for analysis. Stickel *et al*. estimated that the median concentration of heptachlor epoxide in birds on the high dose rate was about 13 p.p.m., although this value should not be taken too literally, because five of the birds were recorded simply as containing more than 300 μg of heptachlor. However, if one takes the lowest possible values, and assumes that no bird contained more than 300 μg of heptachlor epoxide, the mean body content reduces to about 8.9 p.p.m., still three times greater than the concentration in their food.

One considerable uncertainty applies to these concentration factors. The earthworms were preserved in alcohol until analysed, and there is the possibility that some of the heptachlor would have been lost by volatilization when the alcohol was evaporated off. Certainly the earthworms were found to contain very low concentrations compared to the soil (cf Table 4.5.3), although the exposure period was short and the worms were reported to have moved and fed only a little. If the worms had lost about two-thirds of their heptachlor residues to the alcohol, then the actual concentration factor from worm to bird would be 1.0.

A similar feeding experiment, with dieldrin, was made with five individual song thrushes (*Turdus ericetorum*), which were fed for 42 days on a mixture of contaminated and uncontaminated earthworms (predominantly *Lumbricus terrestris*) to give a range of dieldrin concentrations in the birds' diet (Table 4.5.8), and this diet was supplemented with a proprietary bird food (Jefferies and Davis, 1968). It is not known, for either of these experiments, whether steady-state concentrations of pollutant had been established. Nevertheless, some important details can be compared:

(1) Woodcocks consumed on average a weight of food equivalent to about 80% of their body weight per day, about double the rate for song thrushes (Table 4.5.8).
(2) Woodcocks retained, after 60 days on the low dose rate, about 7.9% of the ingested heptachlor residues. Song thrushes, after 42 days, retained, at the most, 4.4% of the ingested dieldrin, and the lower dose rates, comparable for concentration of insecticide in the food to the exposures for woodcock, retained at the most 2.5% of the ingested dieldrin.

In brief, assuming that concentration factors based on dry and wet weights are comparable, concentrations of heptachlor in woodcock were greater than in

Table 4.5.8 Consumption of food and of dieldrin by five song thrushes (*Turdus ericetorum*) fed on a diet with contaminated earthworms (mostly *Lumbricus terrestris*).
Birds A–D were fed for 6 weeks, and then killed for analysis. Bird E died from dieldrin poisoning after 8 days (data from Jefferies and Davis, 1968)

Bird	A	B	C	D	E
Concentration of dieldrin in total diet (based on wet weight) of worms (p.p.m.)	0.15	0.32	3.06	5.69	12.38
Food intake (g/day)	31.6	32.4	29.9	30.5	30.7
Body weight of thrushes (mean of weight at start and end of experiment) (g)	77.9	74.0	73.1	78.3	66.2
Daily food consumption as per cent of body weight	40.5	43.7	40.9	39.0	46.5
Concentration of dieldrin in whole body (fresh weight) (p.p.m.)	0.02	0.09	1.36	4.03	4.27
Concentration factor	0.13	0.28	0.44	0.71	0.34
Per cent of ingested dieldrin retained within body	0.8	1.6	2.5	4.4	8.5

their food, and concentrations of dieldrin in song thrushes were less. Woodcock ate twice as much food per unit of body weight, and retained more of the ingested insecticide: the relative significance of the rate constants for assimilation, excretion and metabolism are unknown.

A similar but less thorough study was made with goshawks (*Accipiter g. gentilis*) fed on chickens contaminated with methyl mercury (Borg *et al.*, 1970). The chickens were fed wheat dressed with 8 p.p.m. methyl mercury for 5–6 weeks, and were then killed. Their muscle and liver tissue was stored, frozen, until fed to goshawks. The total mercury content of these tissues, mostly in the form methyl mercury, was about 10 and 40 p.p.m. respectively. Four hawks were fed on these contaminated tissues, and they started to take less food after the first 10 days, as toxic symptoms developed. The chickens consumed about 0.4–0.5 mg Hg/kg body weight/day, and the goshawks consumed about 0.7–1.2 mg Hg/kg body weight/day.

Thus, 5–6 weeks exposure of chickens to 8 p.p.m. of mercury (9.4 p.p.m. dry weight) yielded 10 p.p.m. of mercury (27 p.p.m. dry weight) in the breast muscle. Goshawks fed on a diet, predominantly of muscle, with about 13 p.p.m. of mercury, for little more than 3 weeks, acquired 40–50 p.p.m. mercury in muscle (Table 4.5.9). These data are incomplete, and, like the previous two studies, there is no indication whether mercury levels were close to a steady-state. However, they do suggest that mercury is retained less readily in chickens than in goshawks. Possibly mercury is absorbed from seed less efficiently than from muscle, and perhaps, like is not being compared with like:

Table 4.5.9 Concentration of mercury in goshawks (*Accipiter g. gentilis*) fed tissue from chickens that had been fed on wheat dressed with methyl mercury (data from Borg *et al.*, 1970)

Concentration of methyl in diet	Survival time	Weight of mercury consumed	Concentration of total Hg (most in form of methyl mercury) (mg/kg wet weight)			Final body weight	Increase in the mean body concentration of total Hg if all Hg retained
(p.p.m. as Hg)	(days)	(mg)	Liver	Kidney	Skeletal muscle	(g)	(mg/kg wet weight)
13	30		114	138	42	960	20.8
	47	20	122	—	45	650	30.8
	38		103	121	52	910	22.0
10	39	18	144	131	39	680	26.5
Controls	46[a]		4.7	—	3.3	1 150	—
	43[a]		0.23	—	0.21	1 580	—

[a] Killed for analysis.

muscle of goshawks contains more myoglobin than muscle of chickens, and myoglobin appears to increase the retention of mercury by muscle tissue. Nevertheless, these data do suggest that mercury increased in concentration, in at least some tissues, along this food chain.

4.5.4.3 Fluctuations in the Degree of Contamination in Food

In all three of these experimental studies, exposures were relatively constant for appreciable periods of time. In field situations, exposures are likely to vary from time to time and from place to place (Davis, 1966), and this can have implications for the interpretation of data.

Jefferies *et al*. (1973) contrasted the very small and insignificant degree of contamination by organochlorine insecticides that is normally found in small mammals, with the extremely high and sometimes lethal levels found in birds that feed on these mammals. This contrast implies that either these birds retain most of the residues that they ingest, or their exposures fluctuates greatly during the year. They therefore trapped wood mice (*Apodemus sylvaticus*) in an arable field for 17 days before, and 13 days after, the field was sown with winter wheat that had been dressed with dieldrin and mercury. The amounts of these contaminants in samples of mice increased, after the wheat had been sown, 68- and 11-fold respectively. Residues in the mice rose throughout the 13 days after sowing, and the maximum recorded individual concentrations were 22.3 and 0.75 μg/g wet weight, compared to mean concentrations before sowing of 0.17 ± 0.025 and 0.04 ± 0.005 μg/g wet weight, increase of 131-fold for dieldrin and 19-fold for mercury.

Apodemus form a large part of the diet of the tawny owl (*Strix aluco*) (Table 4.5.1). Barn owls (*Tyto alba*) and kestrels (*Falco tinnunculus*) also feed on this species. The intake of pesticides by these predatory birds is likely therefore to vary considerably from time to time, and these fluctuations may be magnified if those mice with the highest residues are thereby induced to behave abnormally, which may attract a predator's attention.

Exposure can also vary from place to place. Three species of small mammal, the insectivore *Sorex araneus* (common shrew) and the rodents *Apodemus sylvaticus* (wood mouse) and *Microtus agrestis* (field vole), were sampled at 3–4 sites along a transect of decreasing metal contamination from a derelict mine (Roberts and Johnson, 1978). Analyses of the presumed components of these mammals' diet (see legend to Figure 4.5.3 for details) also enabled estimates to be made of their metal intake (Fig. 4.5.3). There is some evidence of differences between species, although the carnivorous shrew has metal concentrations at least as low as the omnivorous wood mouse and herbivorous vole. There is clearly a difference between metals: zinc levels are regulated, whereas lead levels reflect those in the diet. For cadmium also, levels in these species correlated with levels in the diet. Concentration factors were never appreciably greater than 1.0, and were often considerably less, except for low-level exposures of zinc in the diet of the vole and the wood mouse. In all instances concentrations in both diet and animals were lower than the estimated soil concentrations.

This study does illustrate the difficulties of relating concentrations of pollutant in the body to the exposure. Considerable effort was made to estimate the degree of metal contamination in the diet, but there are inevitably doubts about what the animals actually eat, and also about how far they range to feed: food from different places may be expected to have different degrees of contamination.

In a wider context, animals are not static. Apart from their relatively trivial day-to-day movements, species often have larger-scale movements related to dispersal, season (e.g. migration) and to breeding activities, all of which will tend to give animals irregular exposures to pollutants. This irregularity leads then to the question: how great is the variation between individuals from within the same area?

Some care is needed in answering this question. Differences between individuals, taken for analysis at different times, may result from differences in exposure or from seasonal changes, but analysis of birds' eggs might be expected to minimise variation from seasonal changes. Studies on heron (*Ardea cinerea*) demonstrated the magnitude of the variation that can occur. Eggs from a breeding colony in Troy, Lincolnshire, were analysed for *p,p'*-DDE and for HEOD in 1966, 1968, 1970, 1973 and 1977 (Cooke *et al.*, 1979). After the first occasion the eggs were also analysed for PCBs (Figure 4.5.5). There is clearly a considerable variation between clutches in their content of contaminants. The data for 1968, where an average of four eggs/clutch were analysed, were examined to detail. The range of concentrations within clutches tended to increase linearly with the mean concentration, so analyses of variance were made on the logarithms of the concentrations. Variation in concentrations of DDE and HEOD between clutches was much greater than that within clutches ($F_{22,70}$ = 80.76 and 92.59 respectively, $P < 0.001$). The histograms in Figure 4.5.5 can therefore be taken to indicate real differences in the pollutant content of clutches laid by different birds in the same colony. The pattern of distribution is different both for different pollutants and for different years. An increasing, though small, proportion of eggs contain relatively high levels of PCBs in the later years, whilst the opposite, of an increasing proportion of eggs with low concentrations, occurs for DDE and dieldrin. These differences between years were shown to be significant by a χ^2 test (Table 4.5.10): the range of concentrations was chosen to ensure that no expected frequency was less than 5.0, and the data were corrected for lack of continuity.

The simplest explanation for the observed distributions and changes with time is that a relatively small proportion of birds got a relatively high

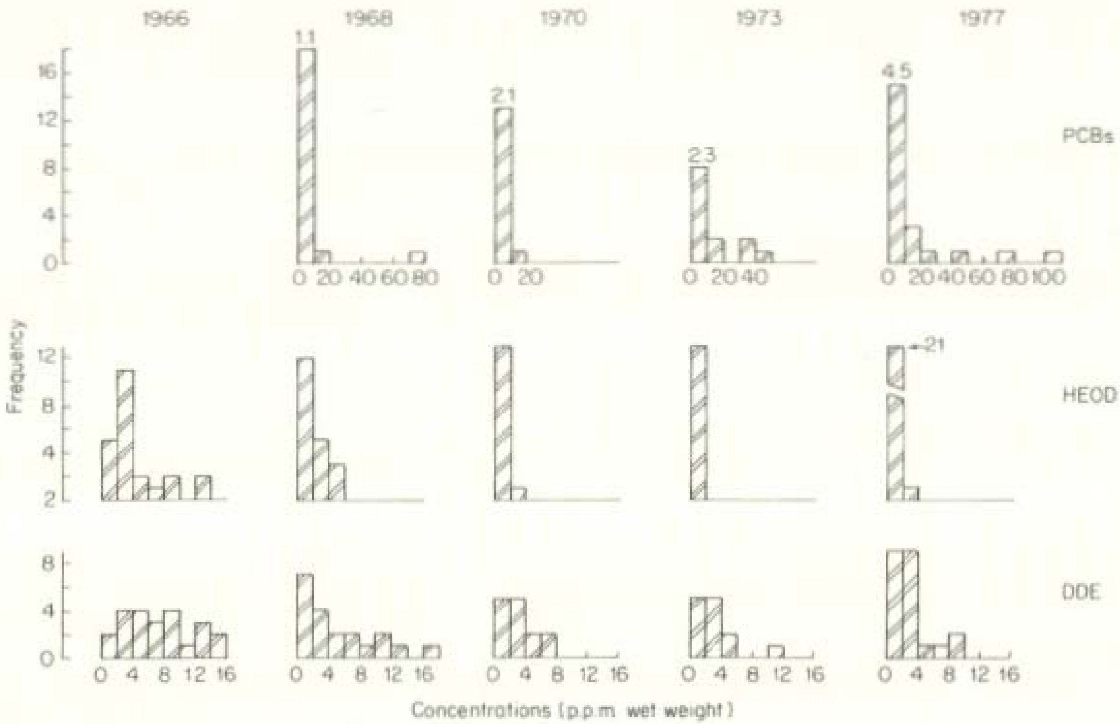

Figure 4.5.5 Frequency histograms for the concentrations of PCBs, HEOD and p,p'-DDE in eggs of the heron (*Ardea cinerea*) taken from a breeding colony at Troy, Lincolnshire. values for 1966 are means based on analyses of 1–5 eggs/clutch (mean 4.0 eggs/clutch). In all other years, rarely was more than 1 egg analysed from a clutch. For PCBs, for values falling within the lowest frequency range, the arithmetic mean concentration is also given. Note that the scale for concentrations of PCBs is five times that for HEOD and DDE (data from Cooke *et al*., 1979)

Table 4.5.10 The significance of the changing proportions of eggs with low concentrations of PCBs, HEOD and DDE in eggs of the heron (*Ardea cinerea*). For further details see Figure 4.5.5

Compound	Concentrations taken as 'low' (p.p.m. wet weight)	X^2	P
PCBs	0–2	11.04	<0.05
HEOD	0–1	44.40	<0.001
p,p'-DDE	0–4	14.47	<0.01

exposure: there is good experimental evidence to show that for dieldrin in the crowned guinea-fowl (*Numida meleagris*), for DDE in the American kestrel (*Falco sparverius*) and for p,p'-DDT in the domestic hen, the concentration in the egg approximates to that in the diet when concentrations are in a steady-state (Wiese *et al*., 1969; Lincer, 1975; Moriarty, 1975). Whether these herons were anywhere near to a steady-state condition is unknown. Herons at the Troy colony disperse over a wider area out of the breeding season, and during the breeding season, whilst based at Troy, each bird has its own particular feeding area, probably within a 20-mile radius (Bell, pers. comm.).

4.5.5 PREDICTION OF BIOACCUMULATION

Most tests proposed or in use as measures of bioaccumulation are for aquatic species. For example, the OECD guidelines for testing of chemicals (1981) list five tests for bioaccumulation, and all five tests use fish. These guidelines then suggest that the *n*-octanol : water partition coefficient gives a good indication of bioaccumulation for terrestrial species. Non-ionized organic chemicals do cross the gut wall by passive diffusion, and therefore the *n*-octanol : water partition coefficient gives a useful measure of one important factor that determines the absorption rate of such compounds (Houston and Wood, 1980). However, other factors, particularly metabolism, also exert a large effect on the degree of bioaccumulation, so the use of partition coefficients alone is inadequate.

We have already discussed the difficulties of interpreting field data. One reaction has been to develop model ecosystems (Metcalf *et al*., 1971), simulation of ecosystems, which are usually relatively small containers with sand, water and a range of species. The compound of interest is introduced into the model ecosystems, and measurements can then be made on the quantities and distribution of both the compound and its metabolites. The 'ecological magnification' and 'biodegradability index' of the compound can then be calculated. However, these quantitative measurements are difficult to interpret (Moriarty, 1977): all that is known is the amount of compound introduced, and amounts in the organisms at one time. Metcalf (1977) also has concluded that these model ecosystems are of limited value, and it is now becoming widely accepted that many years of research would be needed before the relevance of model ecosystems to other systems, natural or artificial, could be assessed (Gillett and Witt, 1979; Pritchard, 1982).

Predictions of the degree of bioaccumulation require two approaches: knowledge of likely pathways taken by the potential pollutant in the environment, which determines the species with the highest exposures, and use of compartmental models in laboratory experiments, with particular emphasis on assimilation and persistence within the body.

4.5.6 SUMMARY AND CONCLUSIONS

Food is the principal source of many pollutants for terrestrial animals. Feeding patterns are not constant: food varies with season, an animal's age and size, and with an animal's location. In practice, it is difficult to determine precisely what an animal does eat, and, therefore, what is its exposure.

Concentration factors are difficult to interpret, but data for heavy metals and organochlorine insecticides show that no universal statement can be made for these contaminants about the increase in concentration, or lack of it, from the abiotic environment into earthworms. There have been virtually no meaningful estimates of concentration factors for vertebrates, in the field. Nevertheless, analyses of field specimens, particularly birds, have led to the suggestion that predators tend to have the highest concentrations of organochlorine insecticides, as a direct consequence of their position in the food chain. In general, successive terrestrial predators are thought to contain successively higher concentrations of persistent contaminants.

The emphasis on trophic level as a determinant of pollutant concentrations appears to be misplaced. The likelihood that a pollutant will increase in concentration along a food chain depends primarily on physiological and biochemical functions within individual animals: the percentage assimilation of an ingested pollutant, and its rate of elimination, i.e. metabolism plus excretion. Trophic level is of significance primarily for its relationship to size: large predators appear less likely than are small predators to have concentration factors greater than 1.0. More detailed information about environmental pathways is needed before amounts in individual food webs can be assessed. Field exposure is likely to fluctuate or to be intermittent, which will complicate such assessments.

It follows that, to predict the likelihood of bioaccumulation of a chemical, two measurements are needed: the percentage assimilation, and the degree of persistence within animals. These are best measured in standardized laboratory conditions.

Predictions of the magnitude of bioaccumulation require two types of knowledge: first, detailed knowledge of the environmental pathways of a potential contaminant that indicates the species that would get the highest exposure; secondly, laboratory measurements of amounts within organisms during and after exposure to assess the rates of assimilation and loss.

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