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**A model for environmental risk assessment and
standard setting based on biomagnification.
Top predators in terrestrial ecosystems.**

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SUMMARY

Soil contaminants accumulating through food chains may exert toxic effects on birds and mammals (secondary poisoning). In standard setting procedures for soil contaminants attention has to be given to these effects next to direct effects on soil organisms. Romijn et al. (1991b) presented an algorithm for risk-assessment on secondary poisoning of birds and mammals; $MPC_{soil} = NOEC_{species\ of\ concern} / BAF_{food\ of\ species\ of\ concern}$ in which: MPC_{soil} is the maximum permissible concentration for a chemical in the soil, $BAF_{food\ of\ species\ of\ concern}$ is the bioaccumulation factor representing the ratio between the concentration in the food of the species of concern and the concentration in soil. The $NOEC_{species\ of\ concern}$ is the no-observed effect concentration. In the current procedure for setting soil quality objectives, MPCs for secondary poisoning are based solely on the food chain *soil* → *worm* → *bird/mammal*.

In the present study a model is developed to estimate MPCs for soil contaminants based on the risk of secondary poisoning for top predators. The above-mentioned algorithm is extended by:

1. taking into account the major terrestrial food chains
2. applying correction factors to the NOECs to account for differences between laboratory and field conditions.
3. generating probability distributions for MPCs by treating BCFs, BAFs and NOECs as stochastic variables.

A simplified food web with three trophic levels is designed: plants and invertebrates at the first, small birds and mammals at the second, and birds and beasts of prey at the third trophic level. Exposure of top predators via separate food chains is analyzed first. However, under natural conditions most top predator species are exposed via more than one food chain (food web). Therefore, a species specific approach is followed, for which eight bird of prey species and two beast of prey species with different food choice are selected: Sparrowhawk, Goshawk, Buzzard, Kestrel, Long-eared Owl, Tawny Owl, Barn Owl, Little Owl, Badger and Weasel.

Six compounds are selected based on the availability of bioaccumulation and toxicity data: DDT, dieldrin, lindane, pentachlorophenol (PCP), cadmium and methyl mercury.

Model calculations are made with average values for food choice and correction factors. The model can be adjusted to specific locations, seasons and life-stages by varying these input parameters.

Literature data reveal that it is appropriate to correct for caloric content and assimilation efficiency of food types, as well as for metabolic rate of birds and

mammals. The scarce information about pollutant assimilation efficiency and species sensitivity does not indicate the necessity to correct for these factors.

The most critical food chains for secondary poisoning of top predators (lowest MPCs) are *soil → insect → bird → bird of prey* for DDT and dieldrin, *soil → insect → bird/mammal → bird of prey* for lindane, *soil → worm → mammal → beast of prey* for PCP and methyl mercury, *soil → worm → bird/mammal → bird of prey* for Cd. The risk for the selected top predator species is much lower than the risk based on these critical food chains, because the critical food chains constitute a minor part (21% at maximum) of their food webs. Species feeding on birds (Sparrowhawk, Goshawk) and small carnivorous mammals (Buzzard, Barn Owl) are exposed to a much higher extent to the contaminants than species feeding on small herbivorous mammals (Kestrel, Long-eared Owl).

MPCs calculated with the method proposed in this report are compared for top predators and worm-eaters. The food chain *soil → worm → bird or mammal* is the most critical food chain for Cd and PCP. The MPCs derived with this chain are low enough for the protection of all selected top predator species in the case of dieldrin, lindane and methyl mercury, but not for DDT.

The model proposed in the present report estimates lower MPCs than the current procedure for secondary poisoning in setting soil quality objectives. Although, the integrated MPCs for the selected chemicals (in: van de Plassche, 1994), based on both (in)direct ecotoxicological effects and equilibrium partitioning, are still low enough for protection of the selected top predator species.

The following recommendations can be made for procedures for derivation of environmental quality objectives based on the risk of secondary poisoning:

1. The food chain *soil → worm → bird/mammal* can be used for terrestrial ecosystems. In case of persistent and highly lipophilic compounds, attention should be paid to top predators, especially birds, exposed through the pathways *soil → worm and insect → birds → top predator*. It should be realized that for most chemicals, the risk-analysis for top predators is seriously hampered by a lack of QSARs and experimental data on bioaccumulation in invertebrate and vertebrate species.
2. Corrections should be applied for differences between laboratory and field conditions concerning metabolic rate, caloric content and assimilation efficiency of food types. The correction for assimilation efficiency is quantitatively much less important as compared to corrections for metabolic rate and caloric content.
3. NOECs, BCFs and BAFs should be used as stochastic variables when possible, providing valuable information about the variation in the calculated MPCs.

SAMENVATTING

Bodemverontreinigende stoffen kunnen via accumulatie in voedselketens toxische effecten hebben op vogels en zoogdieren (doorvergiftiging). Bij de normstelling voor bodem moet rekening worden gehouden met deze effecten, naast de directe effecten op bodemorganismen. Romijn et al. (1991b) presenteerden een algoritme waarmee het risico van doorvergiftiging voor vogels en zoogdieren wordt betrokken in de normstelling; $MTR_{\text{bodem}} = NOEC_{\text{aandachtsoort}} / BAF_{\text{voedsel van aandachtsoort}}$ waarbij: MTR_{bodem} de maximaal toelaatbare concentratie voor een chemische stof in de bodem is, $BAF_{\text{voedsel van aandachtsoort}}$ de bioaccumulatiefactor is, voorstellende de ratio tussen de concentratie in het voedsel van de aandachtsoort en de concentratie in de bodem, en $NOEC_{\text{aandachtsoort}}$ is de hoogste concentratie in het voedsel waarbij nog geen effect optreedt.

In de huidige procedure voor normstelling worden de MTRs in bodem voor doorvergiftiging alleen gebaseerd op de voedselketen: *bodem* → *worm* → *vogel/zoogdier*.

In het onderhavige onderzoek is een model ontwikkeld voor de afleiding van MTRs gebaseerd op het risico van doorvergiftiging voor toppredatoren. Het bovengenoemde algoritme is uitgebreid met:

1. de belangrijkste terrestrische voedselketens
2. correctiefactoren voor de NOECs met betrekking tot verschillen tussen laboratorium en veld omstandigheden.
3. het genereren van een kansverdeling voor de MTR uit stochastische, in plaats van constante, BCFs, BAFs en NOECs.

Een eenvoudig voedselweb met drie niveaus is geconstrueerd: planten en ongewervelden op het eerste, kleine vogels en zoogdieren op het tweede, en roofvogels en roofdieren op het derde trofische niveau. Voor de risico-analyse voor toppredatoren zijn twee benaderingen gevolgd. De blootstelling van toppredatoren via afzonderlijke voedselketens is eerst geschat. De meeste toppredatorsoorten in het veld worden echter blootgesteld via meerdere voedselketens (voedselweb). Daarom is ook gekozen voor een soortspecifieke benadering, waarvoor acht soorten roofvogels en twee soorten roofdieren met onderling verschillende voedselkeuze zijn geselecteerd: sperwer, havik, buizerd, torenvalk, ransuil, bosuil, kerkuil, steenuil, das, en wezel.

Zes stoffen zijn geselecteerd op basis van de beschikbaarheid van gegevens voor bioaccumulatie en toxiciteit: DDT, dieldrin, lindaan, pentachlorofenol (PCP), cadmium en (methyl)kwik.

Modelberekeningen zijn uitgevoerd met gemiddelde waarden voor voedselkeuze en correctiefactoren. Het model kan aangepast worden aan specifieke locaties, seizoenen

en levensstadia door deze inputparameters te variëren.

Uit literatuurgegevens kan afgeleid worden dat er gecorrigeerd moet worden voor calorische waarde en assimilatie-efficiëntie van voedseltypen, en bovendien voor metabolische snelheid (energieverbruik) van vogels en zoogdieren. Van de assimilatie-efficiëntie van stoffen en van soortsgewoetheid is erg weinig bekend. De beschikbare informatie geeft geen aanleiding om te corrigeren voor deze beide factoren.

De meest kritische voedselketens voor doorvergiftiging van toppredatoren (laagste MTRs) zijn: *bodem* → *insekt* → *vogel* → *roofvogel* voor DDT en dieldrin, *bodem* → *insekt* → *vogel/zoogdier* → *roofvogel* voor lindaan, *bodem* → *worm* → *zoogdier* → *roofdier* voor PCP en methyalkwik, *bodem* → *worm* → *vogel/zoogdier* → *roofvogel* voor Cd. Het risico voor de geselecteerde toppredatorsoorten is veel lager dan het risico gebaseerd op deze kritische voedselketens, omdat de kritische voedselketens slechts een klein deel uitmaken (maximaal 21%) van de betreffende voedselwebben. Soorten die zich voor een belangrijk deel voeden met vogels (sperwer, havik) en kleine carnivore zoogdieren (buiserd, kerkuil) worden in grotere mate blootgesteld dan de soorten die zich bijna uitsluitend voeden met kleine herbivore zoogdieren (torenvalk, ransuil).

MTRs voor toppredatoren zijn vergeleken met MTRs voor wormenetende vogels en zoogdieren, berekend met de in dit rapport voorgestelde methode. De voedselketen *bodem* → *worm* → *vogel/zoogdier* is de meest kritische voedselketen voor Cd en PCP. De met deze keten afgeleide MTRs zijn laag genoeg voor de bescherming van alle geselecteerde toppredatorsoorten in geval van dieldrin, lindaan en methyalkwik, maar niet voor DDT.

Toepassing van het model in het onderhavige rapport resulteert in vergelijking met de tot nu toe geldende procedure voor doorvergiftiging in de normstelling tot lagere MTRs. De huidige MTRs voor de selecteerde stoffen, gebaseerd op zowel (in)directe ecotoxicologische effecten als de evenwichtspartitiemethode (in: van de Plassche, 1994), blijken toch laag genoeg te zijn voor de bescherming van de geselecteerde toppredatorsoorten.

De volgende aanbevelingen kunnen worden gedaan met betrekking tot de huidige procedure voor afleiding van normen op basis van het risico van doorvergiftiging:

1. De voedselketen *bodem* → *worm* → *vogel/zoogdier* gebruikt kan worden voor de risicoschatting van doorvergiftiging. Daarnaast moet bij persistente en sterk lipofiele stoffen aandacht worden gegeven aan toppredatoren, vooral vogels, blootgesteld via de routes *bodem* → *worm* en *insekt* → *vogel* → *toppredator*. De risico-analyse voor toppredatoren wordt voor de meeste stoffen echter bemoeilijkt door een gebrek aan QSARs en experimentele gegevens voor bioaccumulatie in zowel ongewervelde als gewervelde dieren.
2. Correcties moeten worden aangebracht voor verschillen tussen laboratorium- en

veldomstandigheden met betrekking tot metabolische snelheid, calorische waarde en assimilatie-efficiëntie van voedseltypen. De correctie voor assimilatie-efficiëntie is kwantitatief veel minder belangrijk dan de correcties voor metabolische snelheid en calorische waarde.

3. NOECs, BCFs, BAFs moeten wanneer mogelijk als stochastische variabelen worden gebruikt, hetgeen waardevolle informatie over de variatie in de MTR oplevert.

1. INTRODUCTION

Soil and water quality criteria are formulated in order to protect among others terrestrial and aquatic ecosystems. These criteria have to be based on both direct exposure of organisms and on indirect exposure via contaminated food, which is called secondary poisoning. Currently secondary poisoning of birds and mammals is included in the standard setting in The Netherlands by considering 3 simple food chains: birds and mammals eating either earthworms, or fish, or bivalves (Van de Plassche, 1994). Attention should also be paid to birds and beasts of prey. Theoretically these organisms may be especially vulnerable to secondary poisoning as they stand at the highest trophic levels in food chains. In the fifties and sixties, top predators were killed by exposure to pesticides like dieldrin and organo-metals like methyl mercury (Koeman et al., 1969). Prolonged decline of Sparrowhawk (*Accipiter nisus*) and Peregrine Falcon (*Falco peregrinus*) populations were attributed to chronic exposure to DDE causing eggshell thinning (Ratcliffe, 1980; Opdam et al., 1987). Accumulation of chemicals in food chains strongly depends on the lipophilicity (K_{ow}) and the capacity of the organisms to detoxify the chemical.

Romijn et al. (1991b) developed a method to assess the risk of secondary poisoning for terrestrial organisms and used the food chain *soil* → *worm* → *worm-eating birds/mammals* as an example. A maximum permissible concentration (MPC) in the soil can be calculated with laboratory data for no observed effect concentrations (NOEC) of birds and mammals and bioconcentration factors (BCF) for earthworms. This is described by the algorithm: $MPC_{soil} = NOEC_{bird/mammal} / BCF_{worm}$. Analogous to this, water quality criteria were estimated with the algorithm $MPC_{water} = NOEC_{bird/mammal} / BCF_{fish}$ (Romijn et al., 1991a, 1993). The Health Council (1993) concluded that the RIVM method offers a pragmatic approach for screening the potential of secondary poisoning of chemicals in birds and mammals. However, the uncertainty in setting quality criteria is high, due to the limited data available in the literature and the limited number of food chains considered. There are indications that the protection of mammals and birds is underestimated with the RIVM method even when correction factors for caloric content of food and for metabolic rate are included (Everts et al., 1992). The Health Council concluded that more research should be done with respect to the accuracy of estimating the exposure concentrations of contaminants in ecosystems. Luttik et al. (1992) made suggestions for investigating the importance of several potentially important correction factors which can be included in the risk assessment for birds and mammals in food webs. They proposed to expand the model of Romijn et al. (1991b) with several correction factors that may influence exposure and sensitivity of vertebrates and therefore may be used to extrapolate from laboratory conditions to field conditions:

1. metabolic rate (energy expenditure) under normal and extreme conditions

2. caloric content of food
3. food assimilation efficiency
4. pollutant assimilation efficiency
5. specific sensitivity of groups of birds and mammals

Moreover they proposed to regard variables as stochasts instead of constant values thereby including the variation in MPCs of contaminants in the soil. In this report this design is worked out.

It was decided to build a generally applicable model for avian and mammalian top predators for six compounds (DDT, dieldrin, lindane, pentachlorophenol (PCP), cadmium and methyl mercury) aimed at deriving soil quality criteria for chemicals based on the risk of secondary poisoning. Protection levels for top predators can be based on exposure via one food chains, for example the most critical one. However, this may be not realistic. Therefore the food webs of several top predator species with different food choice are analyzed too.

The main goal of this research is to come to recommendations for improvement of the reliability of procedures for derivation of environmental quality objectives based on the risk of secondary poisoning. It tried to elucidate which of the proposed extensions of the Romijn algorithm (more and longer food chains, correction factors, variation in both bioaccumulation and toxicity) should be applied.

2. METHODOLOGY

2.1 Diets

There exists a wide variation in food choice among both bird and beast of prey species. The food webs leading to these species may therefore differ considerably. Eight raptorial bird species (four diurnal birds of prey and four owls) and two beasts of prey (Table 1) were selected on the basis of differences in diets, implying differences in exposure to contaminants. These birds and mammals are part of the Dutch (avi)fauna.

Table 1: Selected raptorial species

Common name	Scientific name	Abbreviation
<u>Birds of prey</u>		
Sparrowhawk	<i>Accipiter nisus</i>	sp
Goshawk	<i>Accipiter gentilis</i>	go
Buzzard	<i>Buteo buteo</i>	bu
Kestrel	<i>Falco tinnunculus</i>	ke
Long-eared Owl	<i>Asio otus</i>	lo
Tawny Owl	<i>Strix aluco</i>	ta
Barn Owl	<i>Tyto alba</i>	ba
Little Owl	<i>Athene noctua</i>	li
<u>Beasts of prey</u>		
Weasel	<i>Mustela nivalis</i>	we
Badger	<i>Meles meles</i>	bg

Raptors predate upon a large number of species of small avian and mammalian species. Luttik et al. (1992) proposed to construct a simple food web by lumping small birds and mammals in three groups: herbivores, omnivores and carnivores, but this did not prove to be suitable for the construction of a quantitative exposure model. These three groups are not homogeneous because in each group, many species among themselves differ considerably in the dietary portion of both vegetative and animal food items. Therefore it was chosen to use the weighted food items (groups of invertebrates and plant parts) of small birds and mammals.

In case only (partly) qualitative diets are available the following assumption are made:

1. 75% is used for food item(s) referred to as main food item(s)

2. In case several food items are given either the same qualification or qualifications are absent, equal quantitative portions are assigned to these food items.

At least four plant parts and seven invertebrate groups can be distinguished, being quantitatively important food items for small birds and mammals. For plants a distinction can be made between leaves, seeds, fruits and tubers. The group of invertebrates comprises earthworms, gastropods, larvae of insects, caterpillars, insects (adult), isopods and spiders.

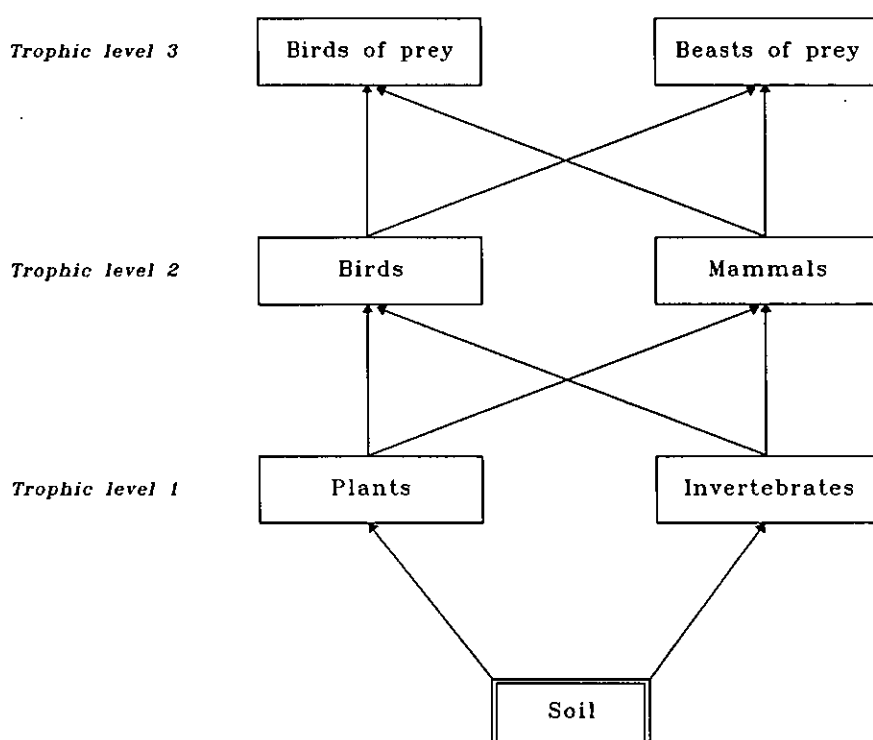


Figure 1. Scheme of a terrestrial food web used for modelling bioaccumulation. The compartments plants and invertebrates can be split up in several groups depending on the availability and variation among bioaccumulation data.

This is the most differentiated form of the food web used in this report. It is used only for cadmium because for this chemical sufficient BCF values were available for each step in this food web. The food web can be simplified by lumping groups of plant parts and by lumping invertebrate groups in a way that makes it suitable for defining the bioaccumulation of chemicals with less data available. Furthermore it

was chosen to construct the food web according to three trophic levels (see Figure 1). It should be realized that in some food chains more trophic levels can be distinguished. For example the food chain *pine tree* → *aphid* → *spider* → *thrush* → *hawk*, with successively a primary producer, primary consumer (herbivore), secondary consumer (carnivore), tertiary consumer (carnivore) and quaternary consumer (carnivore), respectively.

2.2 Method for calculation of MPCs in soil

The MPC_{soil} based on the risk of secondary poisoning for birds and mammals is calculated with a equation first applied by Romijn et al. (1991a):

$$MPC_{soil} = \frac{NOEC_{species\ of\ concern}}{BAF_{food\ species\ of\ concern}} \quad (2-1)$$

in which:

MPC_{soil}	Maximum permissible concentration of a chemical in the soil [$mg_{chem}/kg_{dry\ soil}$]
$NOEC_{species\ of\ concern}$	No-observed effect concentration [$mg_{chem}/kg_{wet\ food}$] corrected for the species of concern
$BAF_{food\ species\ of\ concern}$	Bioaccumulation factor [$kg_{dry\ soil}/kg_{wet\ food}$], representing the ratio between the concentration in food of species of concern (wet weight) and the concentration in soil (dry weight)

For the derivation of an MPC_{soil} from secondary poisoning of top predators new procedures are followed. These procedures are depicted in a scheme (Figure 2) and can be summarized as follows:

1. NOECs from laboratory experiments are separately corrected to NOECs for top predators ($NOEC_{species\ of\ concern}$) by application of the corresponding correction factors, using equation (2-2).
2. A Log-logistic distribution of NOECs for top predators is constructed using the corrected NOECs on the condition that at least four NOECs are available. In case three or less NOECs are available an NOEC value is extrapolated with the modified EPA method and this value is used to derive an MPC_{soil} (see section 2.3).
3. Log-logistic distributions are defined for all BCFs and BAFs as far as possible.
4. With equation (2-1) using the Monte Carlo simulation method (see section 2.2.3) in the computer program Mathcad (MathSoft, 1991) a distribution of MPCs in soil is generated.
5. The 50th percentile (MPC_{50}) and 5th percentiles (MPC_5) of the MPC_{soil}

distribution are calculated, corresponding with a probability of 50% and 5%, respectively, that the NOEC of the species of concern is exceeded. The contaminant concentrations at the 50th and 5th percentiles can be seen as concentrations with a risk of 50% and 5%, respectively. The 5th percentile is arbitrarily chosen as an acceptable level for the protection of individuals of the species of concern, analogous to the van Straalen and Denneman concept (1989) for protection of species within an ecosystem.

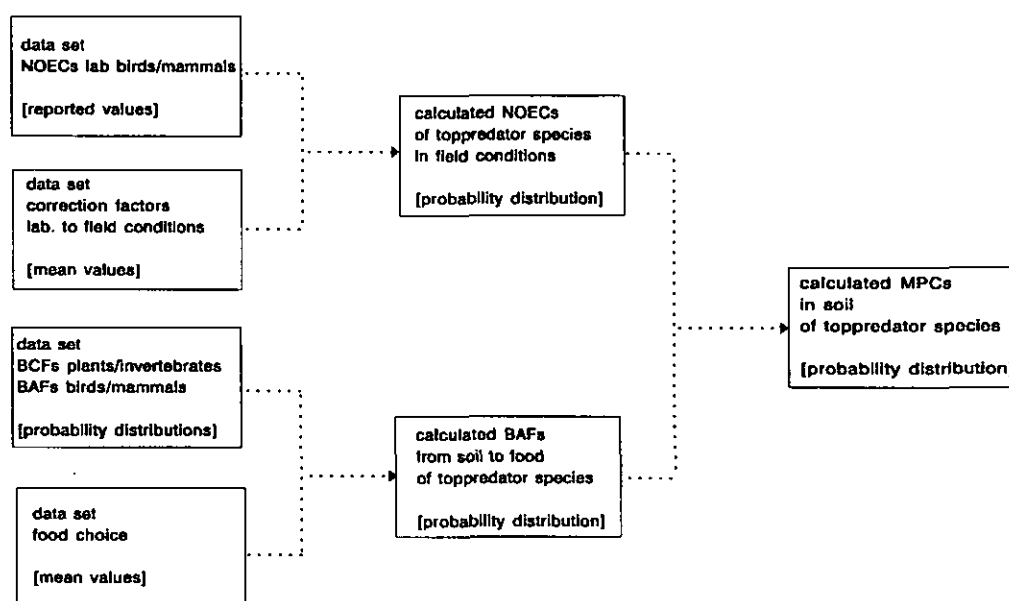


Figure 2. Scheme of the method used for the derivation of MPCs based on the risk of secondary poisoning of top predators. Distributions instead of mean values can be used as input for food choice and correction factors.

2.2.1 NOECs corrected for top predators

An NOEC for a top predator species in field conditions is derived from an NOEC of a laboratory experiment by correcting for differences in caloric content and assimilation efficiency of food, metabolic rate, pollutant assimilation efficiency and

species sensitivity if necessary. This is described by equation (2-2). NOECs for a top predator species can be used to calculate the MPCs of the chemical in the soil by applying equation (2-1).

The NOEC applied to top predators in field conditions ($NOEC_{\text{species of concern}}$) is calculated with:

$$NOEC_{\text{species of concern}} = NOEC_{\text{lab}} * \frac{EMR}{FMR} * \frac{CC_{\text{field}}}{CC_{\text{lab}}} * \frac{FAE_{\text{field}}}{FAE_{\text{lab}}} * \frac{PAE_{\text{lab}}}{PAE_{\text{field}}} * \frac{SS_{\text{lab}}}{SS_{\text{field}}} \quad (2-2)$$

in which:

$NOEC_{\text{species of concern}}$	No-observed effect concentration corrected for top predator of concern
$NOEC_{\text{lab}}$	No-observed effect concentration of laboratory birds or mammals
EMR	Existence metabolic rate [kJ/d]; the energy demand of laboratory animals (see section 4.2)
FMR	Field metabolic rate; the energy demand of free living animals (see section 4.2)
CC_{lab}	Caloric content [kJ/g _{wet food}] of laboratory food (see section 4.3)
CC_{field}	Caloric content of food items preyed upon in the field (see section 4.3)
FAE_{lab}	Food assimilation efficiency [unitless], the proportion of the energy content of laboratory food assimilated by the test species (see section 4.4)
FAE_{field}	Food assimilation efficiency, the proportion of the energy content of the food assimilated by the species of concern in the field (see section 4.4)
PAE_{lab}	Pollutant assimilation efficiency [unitless], the proportion of the pollutant content in the laboratory food assimilated by the test species (see section 4.5)
PAE_{field}	Pollutant assimilation efficiency, the proportion of the pollutant content of the food assimilated by the species of concern in the field (see section 4.5)
SS_{lab}	Sensitivity of test species for the pollutant [e.g. MFO activity relative to the male rat (unitless)] (see section 4.6)
SS_{field}	Sensitivity of species of concern for the pollutant in the field (see section 4.6)

2.2.2 Bioconcentration and bioaccumulation

To indicate the transfer of chemicals in food chains both bioaccumulation factors (BAFs) and bioconcentration factors (BCFs) are used. The following terminology is used according to Moriarty (1983):

BAF defined as the ratio of the test chemical concentration in (a part of) an organism (e.g. bird, mammal) to the concentration in its food (e.g. laboratory fodder, plants, invertebrates, birds, mammals) at steady state. In this report BAFs are used for accumulation by birds and mammals and are expressed on wet weight basis.

BCF defined as the ratio of the test chemical concentration in (a part of) an organism (e.g. plant, earthworm) to the concentration in a medium (e.g. water, soil) at steady state. In this report BCFs are used for plants and invertebrates and are expressed in wet weight tissue and dry weight soil.

Biomagnification phenomenon that a chemical accumulates in species through different trophic levels in a food chain.

The food of top predators generally comprises small birds and/or mammals. Bioaccumulation of chemicals from soil to small birds and mammals takes place in at least two steps, namely a BCF from soil to food (plants and/or invertebrates), followed by a BAF to small birds and mammals.

The exposure of a top predator to a contaminant in the food will depend on the quantitative proportion of participating food items in the dietary routes and the BCFs of these food items. The total bioaccumulation of a contaminant from the soil to the food of a top predator species (BAF_{ft}) can be calculated with equation (2-3).

$$BAF_{ft} = DFT_b \cdot BAF_b + \sum_{k=1}^n (DFB_{ip_k} \cdot BCF_{ip_k}) + DFT_m \cdot BAF_m + \sum_{k=1}^n (DFM_{ip_k} \cdot BCF_{ip_k}) + \sum_{k=0}^n (DFT_{ip_k} \cdot BCF_{ip_k}) \quad (2-3)$$

in which:

BAF _{ft}	total bioaccumulation from soil to the food of a top predator species
BAF _b	BAF of birds
BAF _m	BAF of mammals
BCF _{ip_k}	BCFs of the k th type of invertebrates and plant parts
DFT _b	fraction of birds in diet of top predator
DFT _m	fraction of mammals in diet of top predator
DFT _{ip_k}	fraction of the k th type of invertebrates or plant parts in diet of top predator
DFB _{ip_k}	fraction of the k th type of invertebrates and plant parts in diet of birds
DFM _{ip_k}	fraction of the k th type of invertebrates and plant parts in diet of mammals
n	number of invertebrate and plant parts in the food web of the top predator

2.2.3 Monte Carlo simulations

Both BCFs and NOECs are not constant values but show much variation due to many factors. It is possible to include the variation in calculations with NOECs and BCFs. This is done by describing NOECs and BCFs with log-logistic distributions. With a Monte Carlo simulation technique a probability distribution of MPCs in the soil can be generated. The probability that the NOEC of a species of concern is exceeded at a

given soil contamination level can be calculated. It is chosen to calculate the MPC_5 and MPC_{50} . A comprehensive description of this technique is given in Elbers and Traas (1993).

2.3 Procedures for deriving NOECs of laboratory birds and mammals

Single species toxicity data for the selected chemicals of Romijn et al. (1991b) were updated with single species toxicity data from both reviews and original literature. Analogous with Romijn et al. (1991b) only effects on survival, growth and reproduction were taken into account.

All NOECs from laboratory experiments are corrected according to the procedures in section 2.2.1 in order to be able to derive NOECs for top predators in the field situation. Then these corrected NOECs are used for deriving environmental quality standards. In this report it is proposed to use this approach instead of the method of Aldenberg and Slob (1991). The latter is explained below.

At the moment usually two extrapolation methods are applied in order to derive NOECs for a taxonomic group from single species toxicity data, depending on the number of species for which toxicity data are available:

1. preliminary effects assessment with the modified EPA method (Table 2)
2. refined effects assessment with a method developed by Van Straalen and Denneman (1989) and modified by Aldenberg and Slob (1991)

Table 2. Modified EPA method for birds and/or mammal

Available information	Safety factor
Lowest acute LC50 if less than 3 LC50s and no chronic NOECs are available	1000
Lowest acute LC50 if more than 2 LC50s and no chronic NOECs are available	100
Lowest chronic NOEC if less than 3 chronic NOECs are available	10 *
Lowest chronic NOEC if 3 chronic NOECs are available	10

* this value is compared with the extrapolated value based on acute LC50 values. The lowest value is selected

In case NOECs for more than three species are available the refined method is used, otherwise the preliminary method is used. For species with LC50 or NOEC values for different effect parameters the lowest value is selected. A geometric mean is calculated for species with several LC50 or NOEC values for the same effect parameter.

In the refined method (Aldenberg and Slob, 1991) it is assumed that NOEC values of species within a taxonomic group fit the log-logistic distribution. The 95% level of this distribution is chosen as a reasonable criterium for protection of this taxonomic group in general.

2.4 Procedures for deriving BAFs

2.4.1 Experimental BAFs

The following selection criteria and assumptions were applied:

- 1. BAFs and BCFs were only derived from experiments in which it is either demonstrated or reasonable to assume that concentrations in organisms or plants and in food were in equilibrium.*

It is not always clear whether steady state has been reached, but it is assumed that at relatively long exposure it is valid to derive a BAF from such studies. It should be noted that there are indications that for some chemicals plateau levels in the body will probably not be reached. An example of this is Cd which generally continues to accumulate in birds and mammals. The age distribution of populations of small mammals and birds is important for extrapolation of Cd bioaccumulation data from laboratory experiments to natural conditions. In general many small mammals and small birds do not reach adulthood. A large portion of the prey of birds of prey consists of juveniles. Therefore Cd accumulation studies of a few months may also provide realistic BAFs next to chronic Cd accumulation studies extending over one year.

- 2. BAF and BCF values of toxicants at concentrations causing toxic effects were not considered.*

Toxicity may induce a change in the accumulation process. Moreover, at toxic concentrations, acute toxicity will be used for setting quality criteria and the risk of secondary poisoning can be left out of consideration.

- 3. BCFs in plants and invertebrates were not standardized for soil characteristics.*

Accumulation of chemicals may depend on both soil characteristics (pH, organic matter) and lipid content of organisms but exact mechanisms are not fully understood. In the literature relations between accumulation and soil and compound characteristics are reported for earthworms but not for other invertebrates.

4. *The standardized BCFs for earthworms calculated by Romijn et al. (1991b) will be used.*

Romijn et al. (1991b) concluded that the BCF of Cd in earthworms correlates with the soil-pH and BCFs of organic compounds depend on soil organic matter and lipid fraction of earthworms (section 2.4.2). Romijn et al. (1991b) used a standard soil with pH = 6.5 and 10% organic matter for recalculation of BCFs in earthworms.

5. *A distinction will be made between several invertebrate groups in case there are indications of variation among BCFs of these groups. The same approach is chosen for different plant parts, e.g. leaves, seeds, fruits and tubers.*

In case of a lack of bioaccumulation data two assumptions are made:

- I. BCFs of organic compounds of adult insects, isopods and spiders can be l u m p e d because they are exposed mainly via contaminated food (hard-bodied invertebrates).
- II. BCFs of gastropods, larvae of insects and earthworms can be lumped, because they are soft-bodied soil-dwellers and are mainly exposed via the environment (soft-bodied invertebrates). Caterpillars are also soft-bodied, but not included because they generally are not soil-dwellers.

6. *The litter compartment was left out of consideration, so only BCFs based on concentrations in soil were used.*

BCFs in some invertebrates can be expressed on the basis of soil, litter and food (trophic) concentrations. For instance Cd concentrations in litter are often higher than in the underlying soil, implying that for Cd BCFs on litter basis are often lower than BCFs on soil basis (see Appendix H8).

7. *All invertebrates were positioned in the first trophic level of the model.*

Invertebrates may be clustered in primary (e.g. herbivores), secondary (predators) and tertiary consumers (top predators). However, clustering is sometimes rather arbitrary due a lack of knowledge on feeding strategies. So some invertebrate species stand at the second or third trophic level. It is possible to include food chains like *soil → plant → insect*, and *soil → insect → insect* in the model. However, this option is not chosen for reasons of simplicity and contaminant concentrations of invertebrates are compared to soil concentrations in order to derive BCFs.

8. *Whole body BAFs of birds and mammals were preferred to BAFs for target organs and tissues of these organisms. A total body BAF is estimated with the weighted BAFs of the target organs/tissues when experimental whole body-BAFs are not available.*

Owls completely consume their prey, both mammals and birds. The parts that can not be digested like bones, hair, feathers, bill, etc. are regurgitated. Usually diurnal raptorial birds like Kestrel and Buzzard completely swallow small mammals. However birds serving as prey for Sparrowhawk and Goshawk are plucked and generally only muscles and organs (soft body parts) and some bones are eaten, although muscles and organs/tissues are not always entirely consumed (Brown, 1976; Ratcliffe, 1980). Brown (1976) estimates that 10 to 25% of the total weight of prey animals is not consumed. The top predator is exposed to contaminants in food only by contaminants present in body parts of the prey animal that are consumed and digested. However, the relative contribution of the accumulation of the selected chemicals in nondigestible body parts to the total accumulation is small, with the exception of mercury. Thus it seems appropriate to use BCFs based on whole body basis, and alternatively on the basis of the target organs and tissues including the muscles. The relative weights of the target organs and tissues of the six selected chemicals are presented in Appendix O1. Derivation of BCFs from whole body is preferred due to a relatively high variation in relative weight of the target organs and tissues. In the literature concentrations of metals and organic compounds are often reported exclusively for the target organs/tissues and only rarely for the whole body.

9. *For mammals and birds it was preferred to construct the model with bioaccumulation data from laboratory experiments with terrestrial species. In case these data were lacking, data for raptorial species were used.*

Bioaccumulation data of avian and mammalian species were arranged in five groups:

1. laboratory experiments with terrestrial species (except raptors)
2. laboratory experiments with aquatic species
3. laboratory experiments with raptorial species.
4. terrestrial field data
5. aquatic field data

10. *In the terrestrial model BAFs will be expressed on basis of wet weight tissue of biota and dry weight of soil.*

In the literature concentrations of chemicals in animals, birds and plants are sometimes expressed on a dry weight basis. In that case dry weight-wet weight conversions are made preferably with the dry matter percentages reported in the study itself and when this is not given average dry matter percentages are used (see Appendix P1).

2.4.2 BCFs derived from QSARs

For some taxonomical groups BCFs and BAFs of some chemicals can be estimated with Quantitative Structure Activity Relationships (QSARs). BCFs derived from experiments may differ considerably from BCFs derived from QSARs. Data obtained with QSARs were not used in the model calculations. The available QSARs are shown in order to indicate that they do not offer a reliable alternative for experimental BCFs and BAFs, with the exception of the QSAR for earthworms.

QSARs for plants

Above ground plant (Travis and Arms, 1988):

$$\log BCF = 1.588 - 0.578 * \log K_{ow} \quad (2-4)$$

in which K_{ow} is expressed on dry weight basis

Stems (Briggs et al., 1983):

$$\log SCF = -2.05 + 0.95 * \log K_{ow} \quad (2-5)$$

in which:

SCF, the shoot concentration factor, is the concentration ratio of stem (mg/kg wet weight) and external solution (mg/l).

Roots (Briggs et al., 1982):

$$\log(RCF - 0.82) = -1.52 + 0.77 * \log K_{ow} \quad (2-6)$$

in which:

RCF, the root concentration factor, is the concentration ratio of roots (mg/kg wet weight) and external solution (mg/l).

Concentrations in soil were recalculated to concentrations in the soil solution according to the model used in USES (RIVM, DGM, WVC, 1994):

$$C_{porew_{soil}} = \frac{C_{tot_{soil}} * P_{soil}}{F_{water_{soil}} + F_{solid_{soil}} * K_{p_{soil}} * P_{solid}} \quad (2-7)$$

in which:

$C_{porew_{soil}}$: concentration in the water phase of the soil [$\text{kg}_{\text{chem}}/\text{m}_{\text{water}}^3$]
 $C_{tot_{soil}}$: total concentration in soil [$\text{kg}_{\text{chem}}/\text{kg}_{\text{wet soil}}$]
 P_{soil} : 'bulk density' of the soil [$\text{kg}_{\text{wet soil}}/\text{m}_{\text{wet soil}}^3$]

$F_{\text{water soil}}$: volume fraction of water in soil [m^3/m^3]
$F_{\text{solid soil}}$: volume fraction of solids in soil [m^3/m^3]
$K_{\text{p soil}}$: soil-water partition coefficient [$\text{m}_{\text{water}}^3/\text{kg}_{\text{solids}}$]
P_{solid}	: density of the solid phase in the soil [$\text{kg}_{\text{solids}}/\text{m}_{\text{wet soil}}^3$]

$$K_{\text{p soil}} = \frac{a * F_{\text{oc soil}} * K_{\text{ow}}}{1000} \quad (2-8)$$

in which:

a	: 0.411 as given by Karickhoff (1981) [$\text{m}_{\text{water}}^3/\text{kg}_{\text{oc}}$]
$F_{\text{oc soil}}$: weight fraction organic carbon [$\text{kg}_{\text{oc}}/\text{kg}_{\text{soil}}$]
K_{ow}	: octanol-water partition coefficient [-]

For P_{soil} and P_{solid} values of $1400 \text{ kg}/\text{m}^3$ and $2500 \text{ kg}/\text{m}^3$ respectively were applied and for both $F_{\text{water soil}}$ and $F_{\text{solid soil}}$ 0.4 was used (default values USES, 1994).

QSAR for earthworms

Connell and Markwell (1990) reported a QSAR for BCFs of organic compounds.

$$BCF = \left(\frac{Y_L}{x} * F_{\text{oc}} \right) * K_{\text{ow}}^{b-a} \quad (2-9)$$

in which:

Y_L	: lipid fraction of earthworms (about 0.01)
x	: a constant, estimated to be 0.66 by Rao and Davidson (1980)
$b-a$: b and a are both non-linearity constants: a for the soil to water partitioning and b for the soilwater to earthworm partitioning. Markwell et al. (1989) estimated $b-a$ at 0.07 for earthworms.

It is questionable whether this QSAR can be used also for the estimation of BCFs of organic compounds in invertebrates which are mainly exposed to contaminants via the skin. However, lipid fractions of these animals are rarely determined (Appendix O1). This approach was therefore not followed in this report.

QSARs for vertebrates

Garten and Trabalka (1983) derived the following relationships between BAF and K_{ow} :

For birds in fat:
(with $r^2 = 0.54$)

$$\log BAF = -2.743 + 0.542 * \log Kow \quad (2-10)$$

For mammals (non-ruminants) in fat:
(with $r^2 = 0.35$)

$$\log BAF = -3.849 + 0.617 * \log Kow \quad (2-11)$$

For mammals (ruminants) in fat:
(with $r^2 = 0.34$)

$$\log BAF = -3.935 + 0.511 * \log Kow \quad (2-12)$$

2.5 Literature search methods

In general information was derived from handbooks and reviews which were also used as a starting point for retrospective literature research.

Studies with data on bioaccumulation were searched with the BIOSIS on-line database. Bioconcentration of pp-DDT, pp-DDE, pp-DDD, dieldrin, lindane, PCP, and inorganic-Hg and methyl Hg in terrestrial organisms in the period 1970 to August 1992 and bioconcentration of Cd in terrestrial organisms (without mammals) in the period 1983 to August 1992.

Data on the toxicity of the selected chemicals to birds and mammals reported in Romijn et al. (1991b) were updated. For this purpose CD-ROM with TOXLINE PLUS in the period 1991-June 1993 was consulted.

3. DIETS

Diets of both diurnal raptors and owls are well quantified (Appendix A1, Tables 3,4,5). Average diets on annual basis are used in the model. However, quantitative data on the diet of the Little Owl are only available for the reproductive period (Van Zoest and Fuchs, 1988). This study is chosen, because the proportion of invertebrates is quantified properly. It should be noted that there can be considerable variation in the diet of species depending on the supply of prey which is connected with the habitat as was found for the Barn Owl (De Bruijn, 1979). The Kestrel and Buzzard can be qualified as mainly mammal-eaters, whereas Goshawk and Sparrowhawk are mainly bird-eaters, but these two species generally choose different prey species. The owls are mainly mammal-eaters but show a great interspecific variation in the species they select. For instance the Long-eared Owl strongly prefers herbivorous mammals, whereas carnivorous mammals make up a substantial part of the diet of the Barn Owl.

The diet of the Weasel contains only vertebrates, with mainly mammalian species and also some avian species. On the other hand vertebrates are of less importance to the Badger, which eats mainly vegetable parts and invertebrates, especially earthworms (Table 5).

Quantitative data on the diets of small birds (mainly passerines) and small mammals are not available for all species. The diets of the major prey birds and prey mammals of the selected raptorial birds are shown in Appendices A3 and A4, respectively. An effort is made to quantify the data for four groups of plant parts and seven groups of invertebrates.

For each raptorial bird species a food web can be built with the data of Appendices A5 and A6. Table 3, 4 and 5 show the total picture of the food web with connections between the eleven trophic level 1 compartments (plant parts and invertebrate groups) and the two trophic level 2 compartments (a bird route and a mammal route). This food web is used for the calculation of the bioaccumulation of Cd, because BCF values were available for each step in this food web. The resulting food webs for methyl mercury and for the four organic compounds are shown in Appendices A7 and A8.

Table 3. Food web of selected diurnal bird of prey species¹ (in percentage of total diet on WW basis).

	Sparrowhawk		Goshawk		Buzzard		Kestrel	
	B	M	B	M	B	M	B	M
Leaves	1.0	0.0	20.7	7.5	2.9	50.6	0.0	95.1
Fruits	23.0	0.0	18.1	0.0	0.6	3.2	0.0	0.1
Seeds	36.0	0.0	23.2	0.0	8.9	4.3	0.0	2.9
Tubers	0.0	0.0	20.1	2.5	2.6	6.5	0.0	0.0
Earthworms	8.8	0.0	2.8	0.0	0.3	13.4	0.0	0.6
Gastropods	3.2	0.0	1.0	0.0	0.3	0.4	0.0	0.0
Insect larvae	6.0	0.0	0.9	0.0	0.1	3.4	0.0	0.9
Caterpillars	5.8	0.0	0.9	0.0	0.1	0.0	0.0	0.1
Insects	15.2	0.0	2.4	0.0	1.0	1.3	0.0	0.1
Isopods	0.1	0.0	0.0	0.0	0.1	0.0	0.0	0.0
Spiders	0.8	0.0	0.0	0.0	0.0	0.1	0.0	0.2
Total	100.0	0.0	90.0	10.0	16.8	83.2	0.0	100.0

¹ This is the diet in the most differentiated form in view of the available data. In this form it is used to calculate bioaccumulation of cadmium in food webs.

B = route via birds, M = route via mammals.

Table 4. Food web of selected owl species¹ (in percentage of total diet on WW basis).

	Long-eared Owl		Tawny Owl ²		Barn Owl		Little Owl ²		
	B	M	B	M	B	M	B	M	I
Leaves	0.1	77.3	0.2	24.0	0.0	57.2	0.0	10.4	0.0
Fruits	3.0	1.1	12.2	3.4	0.1	1.8	0.1	1.8	0.0
Seeds	3.6	7.9	4.8	10.4	6.6	8.3	5.0	11.8	0.0
Tubers	0.0	0.0	0.0	1.7	0.0	0.0	0.0	0.0	0.0
Earthworms	1.0	0.0	6.0	10.5	0.0	12.8	0.0	20.8	23.5
Gastropods	0.6	0.0	3.4	0.0	0.0	0.0	0.0	0.0	0.0
Insect larvae	1.5	0.0	0.5	2.6	0.0	3.1	0.0	5.8	0.0
Caterpillars	1.3	0.0	1.2	0.6	0.0	2.8	0.0	0.7	9.0
Insects	1.9	0.7	6.2	1.7	0.4	2.6	0.3	0.7	9.4
Isopods	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Spiders	0.0	0.0	0.0	1.4	0.0	4.3	0.0	0.0	0.0
Total	12.9	87.1	34.6	56.3	7.1	92.9	5.4	51.9	41.9

¹ This is the diet in the most differentiated form in view of the available data. In this form it is used to calculate bioaccumulation of cadmium in food webs.

² Frogs constitute 9.1 and 0.8% of the diet of Tawny Owl and Little Owl, respectively, and are not included in the food web.

B = route via birds, M = route via mammals, I = direct route via invertebrates.

Table 5. Food web of two selected beast of prey species¹ (in percentage of total diet on WW basis).

	Weasel		Badger ²		
	B	M	B	M	P & I
Leaves	4.7	51.2	0.1	4.4	14.0
Fruits	5.1	3.7	0.5	0.2	2.0
Seeds	8.0	8.2	0.4	1.8	19.0
Tubers	4.4	1.5	0.0	0.6	0.0
Earthworms	1.4	3.2	0.3	3.2	27.0
Gastropods	0.7	0.0	0.1	0.0	1.0
Insect larvae	0.8	0.8	0.1	0.8	0.0
Caterpillars	1.2	0.2	0.1	0.2	0.0
Insects	3.1	0.6	0.3	0.3	18.0
Isopods	0.2	0.0	0.0	0.0	0.0
Spiders	0.5	0.5	0.2	0.4	0.0
Total	30.0	70.0	2.0	12.0	81.0

¹ This is the diet in the most differentiated form in view of the available data. In this form it is used to calculate bioaccumulation of cadmium in food webs.

² Amphibians constitute 5% of the diet of the Badger and are not included in the food web.

B = route via birds, M = route via mammals, P = direct route via plant parts, I = direct route via invertebrates.

4. FACTORS INFLUENCING TOXIC EFFECTS

4.1 Introduction

Several correction factors may influence exposure and sensitivity of birds and mammals to contaminants. In this chapter an overview is given of the existing knowledge of successively metabolic rate, caloric content and assimilation efficiency of food items, pollutant assimilation efficiency and species sensitivity. It is discussed whether it is appropriate to derive correction factors which can be used for the extrapolation from birds and mammals under laboratory conditions to birds and beasts of prey under field conditions. The application of these correction factors may improve the reliability of the soil quality standards.

4.2 Metabolic rate of birds and mammals in field and in laboratory

The purpose was to gain better insight into the difference in the energy expenditure of caged birds compared to the energy expenditure of birds and mammals in the field, under normal and extreme circumstances.

We have attempted to determine the energy metabolism of birds and mammals under conditions demanding a high intake, such as during parental care and when living in cold conditions.

Two main approaches have been used to determine the difference between the energy metabolism of laboratory animals and animals in the field. The first approach is to search for a relationship between body weight and energy metabolism for birds and mammals under both laboratory and field conditions. Comparing the two relationships we have attempted to derive a ratio between them, where possible independent of body weight.

There is much literature available concerning allometric relationships between body weight and basal metabolism. Reliable relationships between body weight and field metabolic rate, however, are scarce. Therefore special attention was paid to the confidence intervals of the regressions of basal metabolic rate.

As a second approach, we attempted to find correlations between energy metabolism of individual species under laboratory and field conditions. If a significant correlation exists an extrapolating factor can be used to ensure sufficient protection of animals in the field.

To determine peak metabolic rates in birds and mammals, maximum possible energy metabolism was also investigated.

In Appendix B1 a survey is given of all the relationships between metabolic rates and body weight which are discussed in the following part of this section.

Metabolic rate

Energy metabolism can be described in terms of three levels.

Basal metabolic rate (BMR). This is the energy expenditure of an inactive animal measured in darkness under thermoneutral and postabsorptive conditions. In the literature many BMR data relating to birds and mammals are available. BMR is determined in the laboratory by measuring the hourly O_2 consumption.

Existence metabolic rate (EMR, see equation 2-2). Caged animals (as usually used for toxicity tests) need energy to eat and to digest food. They do not always live in thermoneutrality and show some activity. This energy added to the BMR is called the EMR. EMR is sometimes measured in terms of O_2 consumption, but more often it is determined by calculating the energy content of the food digested, reduced by the energy content of the excreta.

Field metabolic rate (FMR, see equation 2-2). In the field animals need energy for locomotion, foraging, thermoregulation and reproduction. There are various methods to measure the FMR. If food consumption is known, energy intake can be measured as the caloric content of the food. A second method is to estimate the energy cost that is necessary to fulfil all activities and then to add these costs to the BMR. Both methods are rough approximations. To compare FMRs we only use data that were obtained with the 'doubly labelled water' method, whereby an animal is captured and injected with an amount of Deuterium $D_2^{18}O$ or tritiated water $^3H_2^{18}O$. It is then released and recaptured after a few days. After determination of the concentration of the isotopes in blood and of food intake, energy expenditure can be calculated (Williams, 1988).

Morphological and physiological properties such as muscle mass, absorption efficiency and cellular respiration determine the maximum energy expenditure. Inactive animals need energy (BMR) to support this basal system. Therefore a functional relationship between BMR/EMR and FMR is to be expected (Koteja, 1991).

Basal metabolic rate (BMR)

BMR data for many birds and mammals are available from the literature. For both birds and mammals, log BMR is linearly related to log body weight (BW).

The relation between BMR and body weight is described by the allometric equation:

$$\log \text{BMR} = a + b \log \text{BW} \quad (4-0)$$

BMR is expressed in terms of O_2 consumption per time-unit, watt, kilo-calorie/day or kilojoule/day. In this study, energy is expressed in kilojoule/day and body weight in

grams. Allometric equations expressed in other units have been recalculated to the above units.

Many authors have derived allometric equations for the BMR/body weight relationship of birds.

Because there are considerable differences between the energy demand of passerines and nonpasserines, many authors distinguish these groups of birds when calculating regressions.

Lasiewski and Dawson (1967) calculated the following allometric equations:

$$\text{Nonpasserines: } \log \text{ BMR} = 0.349 + 0.723 \log \text{ BW} \quad (4-1)$$

$$\text{Passerines: } \log \text{ BMR} = 0.559 + 0.724 \log \text{ BW} \quad (4-2)$$

Aschoff and Pohl (1970) gave the following equations:

$$\text{Nonpasserines: } \log \text{ BMR} = 0.287 + 0.734 \log \text{ BW} \quad (4-3)$$

$$\text{Passerines: } \log \text{ BMR} = 0.504 + 0.726 \log \text{ BW} \quad (4-4)$$

Gavrilov (1985) derived an allometric equation without distinguishing passerines and nonpasserines (quoted in Daan et al., 1989):

$$\log \text{ BMR} = 0.530 + 0.677 \log \text{ BW} \quad (\text{n: number of species} = 263) \quad (4-5)$$

Ellis (1984) derived an allometric equation for seabirds only:

$$\log \text{ BMR} = 0.419 + 0.721 \log \text{ BW}.$$

Equations for mammals were derived by Kleiber (1961):

$$\log \text{ BMR} = 0.221 + 0.75 \log \text{ BW} \quad (4-6)$$

and by Hayssen and Lacy (1985), with SD as standard deviation:

$$\log \text{ BMR} = 0.318 (\text{SD} = 0.014) + 0.693 (\text{SD} = 0.11) \log \text{ BW} \quad (\text{n} = 293) \quad (4-7)$$

Apart from these allometric equations, derived from large databases, many published equations relate to small groups of birds and mammals, with the aim of calculating the influence of other parameters on the BMR/body weight relationship. It is possible that habitat type, pigmentation, diet and behaviour patterns influence the BMR/body weight relationship. Taxonomic differences could also influence this relationship.

Bennett and Harvey (1987) analyzed the taxonomic level at which the most reliable regressions for birds could be fitted. Equations derived at the family level gave the

best fit with a correlation coefficient of 0.99:

$$\log \text{BMR} = 0.371 + 0.67 \log \text{BW} \quad (n=78) \quad (4-8)$$

The 95 % confidence limits of the slope of the equation were: 0.65 and 0.70.

McNab (1988) suggested that BMR depends on the feeding habits of birds and mammals. Elgar and Harvey (1987), however, argued that differences in BMR thought to be associated with feeding habits could also be based on taxon-dependent differences in energy metabolism.

The exponent (or slope) of the regression, varies from 0.66 to 0.75, although equations derived for selected groups of animals on the basis of taxonomic level or feed habits might show higher values.

Elgar and Harvey (1987) show for mammals that the taxonomic level determines the value of the exponent: the exponent is 0.60 for species within genera but 0.83 for orders within classes. For birds Bennett and Harvey (1987) found the opposite trend: the exponent was 0.82 for species within genera and 0.68 for orders within classes.

For both birds and mammals the value of this exponent has given rise to much discussion (see Heusner, 1987, 1991, among others). The value of 0.66 has been explained by the fact that the surface area of an object is proportional to the volume of the object raised to the power of 0.66. It is assumed that BMR is directly proportional to the loss of heat from the surface of the organism, thus explaining the value of the exponent. McMahon (1973, quoted in Peters, 1983) attempted to explain an observed value of 0.75 by the theory of elastic similarity, by which he compared energy metabolism for organisms represented by cylinders. This explanation is not generally accepted.

Heusner (1991) analyzed the power function for mammals using data from the literature. He disagreed with those authors who assumed that small mammals have lower exponents than large animals. By analyzing data for small mammals only, he calculated an exponent of 0.66. Inclusion of data from large mammals as well resulted in a larger power function. Analyzing the data for large mammals alone again resulted in an exponent of 0.66.

He concluded that the mass exponent of the regression is not the physiological problem. There are differences in the intercepts of the regressions for large and small animals. Data from large mammals are situated higher in the BMR/body weight plane.

The equations given by Heusner are:

small mammals (< 20 kg): $\log \text{BMR} = 0.3135 \text{ (SD= 0.020)} + 0.677 \text{ (SD= 0.008)} \log \text{BW}$ (n=366) (4-9)

large mammals (> 20 kg): $\log \text{BMR} = 0.7295 \text{ (SD= 0.037)} + 0.679 \text{ (SD= 0.009)} \log \text{BW}$ (n=25) (4-10)

Lavigne et al. (1986) found no differences in BMR between marine and terrestrial mammals. According to this author data in the literature suggesting that BMR is higher in marine mammals are unreliable. They were not obtained under thermoneutral, postabsorptive conditions. The only reliable measurements were carried out with seals (Phocidae), the regression being:

$$\log \text{BMR} = 0.621 + 0.87 \log \text{BW}$$

This equation did not differ significantly from that of Kleiber (4-6).

Regressions given in the literature are rarely accompanied by confidence intervals. Hayssen and Lacy (1985) showed that 21% of measured values of BMR in mammals deviated by more than 50% from the calculated equation.

Heusner (1987) plotted the relative frequency of mass-independent metabolism for 163 mammalian species and 190 bird species (Figure 3). He used the power function 0.67. The energy metabolism varied from 2.5-16 ml O₂/hour/g^{0.67} (1.2-7.7 kJ/day/g^{0.67}). For mammals the average energy metabolism was 5.76, and for birds 6.91 ml O₂/hour/g^{0.67} (2.73 and 3.32 kJ/day/g^{0.67}, respectively).

Analyzing the BMR of more than 40 seabirds, Ellis (1984) recognized deviations of 70 - 200 % from values calculated with the equation of Lasiewski (4-1).

Analyzing BMR at a lower taxonomic level, smaller deviations (\pm 50%) were observed (Bennett and Harvey, 1987).

Existence metabolic rate (EMR)

For Nonpasserine birds at 30 °C and 15 hours photoperiod, Ellis (1984) found that the EMR was related to body weight by the regression:

$$\log \text{EMR} = 0.658 + 0.664 \log \text{BW} \quad (\text{SD}= 0.743) \quad (\text{n}=70) \quad (4-11)$$

EMR values have been measured for many birds and mammals and are about 1.5 to 2 x BMR (Kirkwood and Bennett, 1992). To calculate an EMR/BMR ratio, we compared 68 BMR and EMR values of birds from the database of Kendeigh et al. (1977).

The ratio $EMR/BMR = 1.45 \pm 0.57$.

EMR estimates suffer from the same problem as BMR estimates. Additional problems in estimating the EMR from regressions are differences in activity and food assimilation by different species.

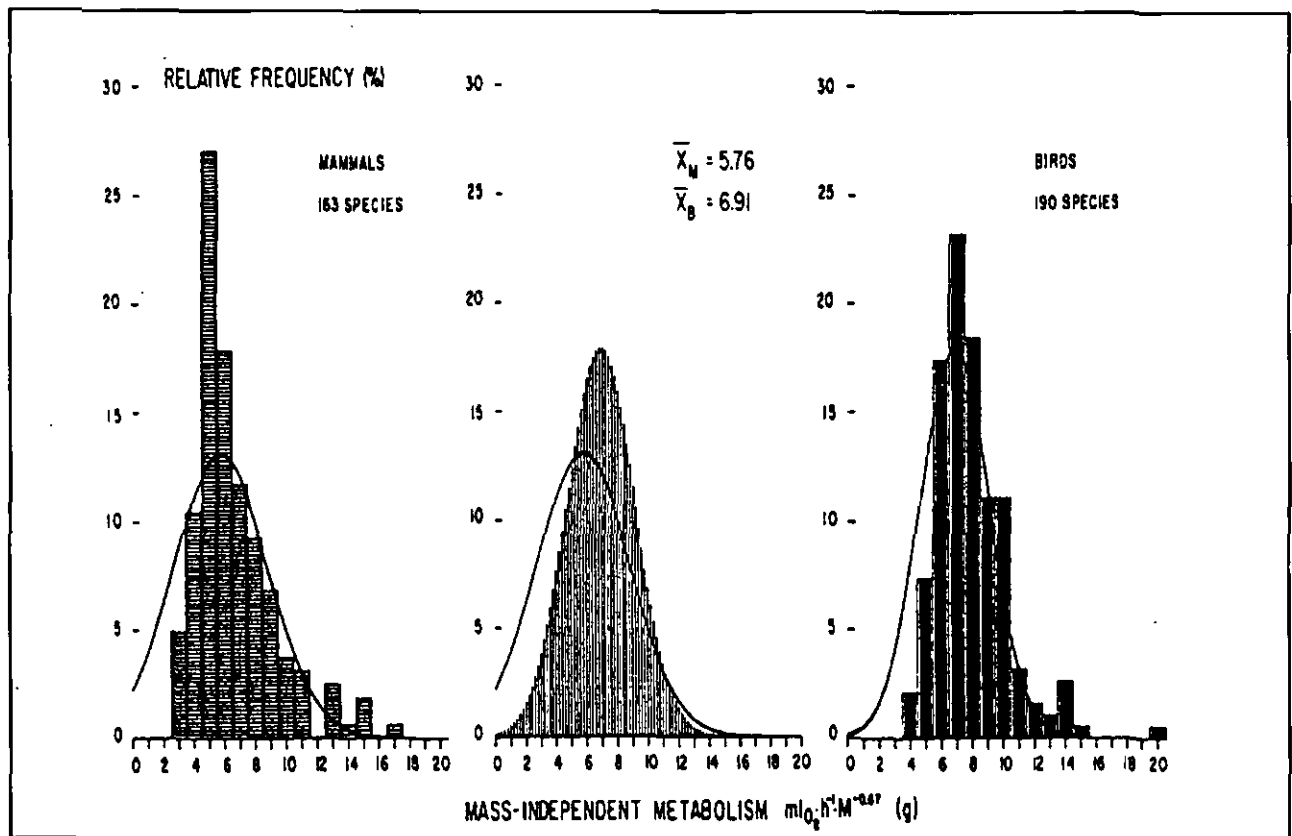


Figure 3. Mass independent energy metabolism (BMR) of mammals and birds. After Heusner (1987).

Field metabolic rate (FMR)

Since the 'doubly labelled water' technique has become available during the last decade, sufficient reliable FMR data have been obtained. Several researchers have derived regressions of FMR and compared them with BMR regressions.

Daan et al. (1990 a,b) analyzed the BMR of 26 bird species and the FMR of 28 breeding bird species. He derived the following regressions:

$$\log BMR = 0.552 + 0.684 \log BW \quad (4-12)$$

$$\log FMR_{par} = 1.140 + 0.659 \log BW \quad par = \text{during parental care} \quad (4-13)$$

The slopes of the BMR and FMR equations were statistically parallel.

Assuming that the slopes of the two equations are equal, the FMR/BMR ratio is constant at 3.9.

Daan et al. (1990b) also analyzed the FMR_{par} of 15 mammal species:

$$\log FMR_{par} = 0.987 + 0.634 \log BW \quad (4-14)$$

The author compared this FMR equation with the BMR equation for eutherians of Hayssen and Lacy (1985):

$$\log BMR = 0.331 + 0.696 \log BW \quad (\text{mammals } n=293) \quad (4-7a)$$

Daan et al. (1991b) considered the slopes of the BMR and FMR equations not significantly different.

If this holds true, the FMR/BMR ratio is 4.5.

Bennett and Harvey (1987) analyzed BMR and FMR data for 47 birds and did not find parallel allometric equations. However, they used FMR data determined with techniques other than the doubly labelled water method. The equations were:

$$\log BMR = 0.431 + 0.68 \log BW \quad (4-15)$$

$$\log FMR = 1.80 + 0.61 \log BW \quad (4-16)$$

The authors pointed out that relative to BMR, small birds use more energy than large birds. they also found that breeding birds, for which they had data, used relatively large amounts of energy compared with nonbreeding birds. In his opinion a constant FMR/BMR ratio cannot be used.

Walsberg (1983) derived the following equation for birds:

$$\log FMR = 1.12 + 0.605 \log BW \quad (4-17)$$

Williams (1988) analyzed FMR data for 38 birds ranging in weights from 4.5 g to 8.4 kg, and derived the allometric equation:

$$\log FMR = 0.982 + 0.661 \log BW \quad (4-18)$$

The FMR of Passerines was a factor 1.8 higher than the FMR of other birds of the

same weight. However, BMR values for these birds were also larger than those for other birds (Williams, 1988).

Koteja (1991) derived the following allometric equations:

Breeding birds:	$\log \text{FMR} = 1.16 + 0.651 \log \text{BW}$	(n=23)	(4-19)
Mammals:	$\log \text{FMR} = 0.885 + 0.613 \log \text{BW}$	(n=37)	(4-20)
eutherians:	$\log \text{FMR} = 0.817 + 0.633 \log \text{BW}$	(n=18)	(4-21)
marsupials:	$\log \text{FMR} = 1.173 + 0.527 \log \text{BW}$	(n=9)	(4-22)

Nagy (1987) also derived allometric equations for birds and mammals.

Mammals			
eutherians:	$\log \text{FMR} = 0.525 + 0.813 \log \text{BW}$	(n=23)	(4-23)
marsupials:	$\log \text{FMR} = 1.072 + 0.576 \log \text{BW}$	(n=13)	(4-24)
Birds:	$\log \text{FMR} = 1.037 + 0.640 \log \text{BW}$	(n=25)	(4-25)
passerines:	$\log \text{FMR} = 0.949 + 0.749 \log \text{BW}$		(4-26)
nonpasserines (mostly seabirds):	$\log \text{FMR} = 0.681 + 0.749 \log \text{BW}$		(4-27)

Eutherian FMR regressions had higher slopes than BMR regressions, while marsupial regressions showed the opposite trend. FMR regression of birds, however, were not significantly different from BMR regressions (Nagy, 1987).

FMR regressions for birds derived by different authors are similar. There are 4 equations with a slope of 0.65 or 0.66 (4-13, 4-18, 4-19 and 4-25). Walsberg (4-17) and Bennett (4-16) found a less steep slope, but they used data obtained with different techniques. The intercepts of the equations for birds derived by Daan (4-13) and Koteja (4-19) were about 20% higher than those of the other two equations with the same slope. This difference in intercepts may be explained by the fact that Daan and Koteja used data for breeding birds only while Nagy (4-25) incorporated other data as well.

From field research, Daan et al. (1989) found that over the annual cycle the Kestrel (*Falco tinnunculus*), expends most energy during parental care. The same has been observed for Dippers (*Cinclus cinclus*). Energy expenditure of Dippers during wintertime is 80 % of this value (Williams, 1988).

Comparing the FMR regressions of Daan et al. (4-13) and Koteja (4-19) with the BMR regression of Gavrilov (4-5) gives, assuming equal slopes, a FMR/BMR ratio of 4.6. Distinguishing between passerine and nonpasserine birds, Williams (1988) and Nagy (1987) derived equations with the same slope but with different intercepts. The ratio between the two regressions (passerines and nonpasserines) was 1.8 and 1.6 respec-

tively. The ratio between the BMR regressions of the two groups is the same at 1.65 (4-1, 4-2; 3-4, 4-4).

Surprising is the fact that separating bird data into the two groups above results in a steeper slope for both BMR and FMR regressions for the two groups compared with the 'pooled' regression. In the literature there is no explanation for this. The reason could be, that passerine birds, being smaller than nonpasserines, have regressions with a larger intercept than nonpasserines. If this is true, adding data from nonpasserines to a database for passerine birds will lower the slope of the regression. This is opposite to what Heusner (1991) describes for mammals where combining data resulted in a steeper regression.

To compare FMR with the BMR equation of Aschoff and Pohl (1970), Masman et al. (1989) analyzed energy expenditure during parental care (FMR_{par}) for nonpasserine and passerine birds separately:

$$\text{Passerines: } \log FMR_{par} = 1.232 + 0.57 \log BW \quad (n=13; \text{average weight } 28.6 \text{ g}) \quad (4-28)$$

$$\text{Nonpasserines: } \log FMR_{par} = 1.245 + 0.62 \log BW \quad (n=19; \text{average weight } 2529 \text{ g}) \quad (4-29)$$

An 'average' bird in the two groups has an energy expenditure (FMR) of 3 to 4 x BMR.

The analysis of FMR in mammals has resulted in equations showing considerable variability.

Nagy derived an FMR equation for eutherians having a steeper slope than the BMR equation (4-23), while Koteja (4-21) found the opposite. For marsupials both authors found a less steep slope in FMR than in BMR regressions (4-22, 4-24). Koteja compared the FMR regression with the BMR regression using data from the same mammals (small number of species), Nagy with the BMR regression of Lasiewski (large number of species).

The intercepts of the regressions are also very different.

However, attention must be paid to the fact that in general the number of data used is small, although the equation for mammals derived by Koteja (4-20) is based on a larger amount of data ($n=37$).

The finding of Koteja did not support the hypothesis of a constant FMR/BMR ratio. He could not find arguments to support the hypothesis that BMR is a reliable index of energy expenditure in the field.

Nagy derived regressions for several groups of eutherian and marsupial mammals and birds. He concluded that within these groups FMR regressions were different from each other for the following subgroups: rodents, passerine birds, herbivorous eutherians, herbivorous marsupials, desert eutherians, desert birds and seabirds.

Differences between birds and mammals

The intercepts of BMR and FMR regressions for mammals are 32% and 37% respectively of those for birds. (Daan et al., 1990b)

Two hypotheses were given by Daan et al. to explain these differences. Firstly there is no fundamental difference between the energy demand of a lactating mammal and that of a breeding bird. Besides its own energy demand, the mammal needs energy to produce milk. The energy for milk production added to FMR, results in the FMR for birds of the same weight. This DME (daily metabolizable energy) would also be equal to the FMR for breeding birds. Daan et al. derived an allometric equation of DME for 15 lactating mammals (mostly laboratory animals):

$$\text{Log DME} = 0.987 + 0.728 \log \text{BW} \quad (4-30)$$

This equation has a steeper slope than the FMR and BMR regressions derived by Daan et al.. This would imply that the energy expenditure of larger lactating mammals is relatively greater than that of smaller mammals. Compared with the equations of Kleiber (4-6) and Hayssen (4-7) however, the slope of the DME equation is not steeper.

Secondly, differences in body composition between birds and mammals are reflected by differences in BMR and FMR. Generally mammals have more metabolically inactive tissue, such as fat deposits, than birds have, and therefore their energy expenditure, calculated per gram body weight, is lower.

Peters (1983) suggested that the metabolism of animals depends on their body temperature. Birds have higher body temperatures than mammals and are supposed to use more energy for thermoregulation. Body temperature ranges are as follows: passerine birds 39-44 °C; nonpasserine birds: 39-41 °C; eutherian mammals 36-39 °C; marsupial mammals: 35-36 °C)

FMR/BMR ratios of individual species

The second way to determine the difference in energy metabolism between animals in the laboratory and animals in the field is to calculate FMR/BMR ratios for individual species. If these ratios generally do not exceed a certain value, this value will indicate an energy level that will not be exceeded by animals under normal circumstances.

Animals with higher BMRs than those calculated from regressions, also seem to have higher FMR values. Daan et al. (1990a) showed this for birds. They calculated a FMR/BMR ratio of 3.57 (± 0.97). The lowest value he found was 1.87, and the highest 5.59.

Koteja (1991) lists FMR and BMR for 52 birds and mammals. The FMR/BMR ratio varies from 1.6 to 6.6, the average value being 3.77. The ratio exceeds 5 only for 8 species. Unfortunately these animals are not a representative sample of all birds and mammals.

Bryant and Tatner (1991) determined the FMR/BMR ratio for 28 species of small birds (10 - 150 g, $n=553$). The ratio varies from 1 to 7 with an average value of 3. The FMR/BMR ratio of 91-94 % was less than 5, that of 97-99 % less than 6.

Some researchers on birds and mammals have pointed out that animals that need a lot of energy to thermoregulate have large FMR/BMR ratios.

Birt-Friesen et al. (1989) working on Gannets (*Sula bassana*) calculated an FMR/BMR ratio of 6.6. This high value was explained by the high cost of thermoregulation (in Newfoundland) and the method of flying.

The FMR of sea mammals was analyzed by Reilly and Fedak (1991). The FMR/BMR ratio of *Phoca vitulina* was 6, being twice as high as the value calculated from Nagy's regression.

Root (1988) investigated the distribution of passerine birds over a large area. It appeared that these birds are restricted in winter to areas where they did not need to raise their energy demand for thermoregulation higher than $2.5 \times \text{BMR}$.

'Sustained metabolic scope' and maximum energy expenditure

From the above it is obvious that there exists no fixed FMR/BMR ratio.

The energy expenditure of breeding birds generally represents the highest value during the annual cycle, but this is not always the maximum possible energy expenditure. Daan (1990b) stated that under natural circumstances, the FMR of birds is barely affected by the number of offspring. In their experiment with kestrels, forced food shortage appeared to increase FMR. A high FMR, however, seems to impose a cost in terms of future survival.

There are costs and benefits for a breeding bird which increases its food intake. Increasing food intake increases the survival of present offspring, but decreases the probability of producing future offspring. There will be an optimum level of energy expenditure.

Several researchers have attempted to determine the optimum or maximum energy expenditure for birds and mammals.

In the laboratory, Novoa et al. (1990) determined the lowest temperature at which the Rufous-collared Sparrow, *Zonotrichia capensis*, can survive. The maximum metabolic costs necessary to resist this non-lethal temperature were 5.30 to $5.71 \times$

BMR.

Peterson et al. (1990) described the maximum energy expenditure that organisms can achieve and the period of time during which this can be sustained. During a few seconds, humans and animals can attain an energy expenditure of about 100 x BMR. This expenditure is fuelled by anaerobic ATP production that cannot be sustained much longer because of the toxic effects of lactic acid build up. Over a longer period aerobically supported energy expenditure can be sustained. The authors suggested that there is a certain level that can be sustained during a relatively long period without using stored reserves. They named this level the 'sustained metabolic rate' (SusMR). Data from birds, humans and other mammals were analyzed and used to calculate SusMR/BMR ratios mostly of 1.5 to 5. There were some outliers, but the ratio was always lower than 7. This level can limit reproductive success, foraging behaviour and the possibility of sustaining low temperatures.

The existence of a SusMR may be explained by the concept that there must be upper limits to the capacity of the digestive tract to process food and to cellular metabolic capacity. This SusMR has to be related to BMR because the metabolic machinery of the animal must have been adjusted by natural selection to the energy requirements during the episode of maximum FMR.

A higher energy demand will enhance physiological costs and increase the probability of death. The SusMR level can be compared with the 'optimal working level' of Daan et al. (1990a).

Kirkwood (1983) performed a literature search for FMR data for animals under energy-demanding conditions such as hard exercise, rapid growth, breeding, lactation or low temperatures. The FMR values appeared to correlate with body weight.

$$\text{Max. Energy expenditure} = 1713 \text{ kJ/kg}^{0.72}$$

$$\text{Log Max. FMR} = 1.070 + 0.72 \log \text{BW} \quad (4-31)$$

The maximum energy expenditure for organisms was between 3 and 6 x BMR. The author calculated that the energy demand to support the metabolic machinery was 500 kJ/kg^{0.72}. The difference between this energy and the maximal energy (1200 kJ/kg^{0.72}) is available for activity, thermoregulation and growth. This corresponds to an FMR/EMR ratio of 2.5.

An advantage to the use of ceiling values of energy expenditure is the fact that it is then not necessary to apply a correction factor for the determination of energy expenditure under more demanding circumstances because ceiling values are maximum values and when an animal needs more energy, it has to use energy from its fat deposits.

For very small birds in the field there seemed to exist a maximum energy expenditure

(Cherel et al., 1988). At low temperatures they required more energy for thermoregulation (6 x BMR) than they could assimilate; under these conditions they entered torpor .

Masman et al. (1989) compared the allometric regression for 30 breeding birds:

$$\log \text{FMR}_{\text{par}} = 1.141 + 0.65 \log \text{BW} \quad (4-32)$$

with the Kirkwood regression. They concluded that FMR_{par} for small birds is equal to maximum FMR, but that for large birds it is only 60 % of the maximal energy expenditure. They hypothesized that because larger species live longer, they are probably more conservative in energy expenditure, having a better chance of survival and therefore of future reproduction.

FMR/BMR ratios of hummingbirds are probably highest of all birds, having values close to 10 (Suarez, 1992).

Taylor et al. (1980) determined the maximal rate of oxygen consumption by mammals. Oxygen consumption, related to lactic acid production, was measured using the treadmill procedure. The weight of the animals varied from 7.2 g to 263 kg. The allometric regression of maximum oxygen consumption (ml/sec) was $1.92 \text{ kg}^{0.79}$ (recalculated as $\log \text{FMR}_{\text{max}} = 1.152 + 0.79 \log \text{BW}$ (4-33)). Maximum consumption was about 10 times the basal metabolic rate. Oxygen consumption seemed not to be related to lung surface but instead to body weight.

Koteja (1987) did not find a constant value of the ratio of maximum energy expenditure/BMR for mammals. Instead FMR regression was steeper than the BMR regression:

$$\log \text{BMR} = 0.342 + 0.745(\text{SD}=0.021)\log \text{BW} \quad (4-34)$$

$$\text{Maximum energy expenditure: } \log \text{FMR}_{\text{max}} = 1.043 + 0.841(\text{SD}=0.033)\log \text{BW} \quad (4-35)$$

The ratio $\text{FMR}_{\text{max}}/\text{BMR}$ varied between 4 and 30. A disadvantage on the study of Koteja is that all data were taken from the literature and that often BMR and FMR_{max} data came from different investigations.

Discussion

Allometric regressions of BMRs for birds and mammals are calculated from large databases. It is not possible to derive a BMR for a bird or mammal of a given weight from allometric regressions with a high degree of reliability. Using allometric

regressions calculated from data for smaller taxonomic units, the BMR of animals belonging to a particular taxonomic group can be derived with greater reliability.

An explanation of deviations from calculated regressions is given by the large variation in the anatomy of animals, for example in the relative proportions of organs, muscle and fat. Different tissues consume different amounts of energy.

The O_2 consumption of different tissues from Starling (*Sturnus vulgaris*) and Dunlin (*Calidris alpina*) has been determined. Relative to weight, fat tissue consumes only 10% of energy compared with muscle tissue and 2% compared with liver tissue. Comparing data relating to the energy consumption of tissues, bird tissue consumes more energy than mammalian tissue (Scott and Evans, 1992).

Daan et al. (1990a) calculated that BMR of birds correlated better with the weight of the heart and kidney than with total body weight. Heart and kidney consume much more energy than other organs and tissues.

The reliability of allometric regressions will be improved, if, in future, more BMR data related to heart and kidney weights become available.

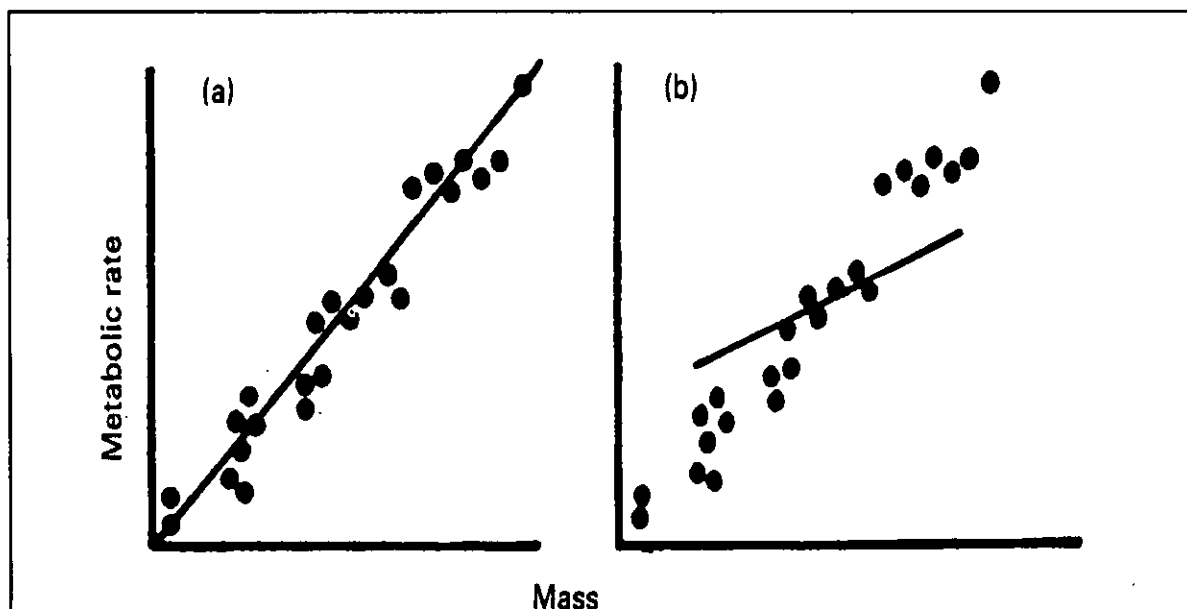


Figure 4. Calculation of the regression between metabolic rate and body weight for groups of animals with a wide (a) and with a narrow (b) range of body weight (from: Kirkwood and Bennett, 1992)

Recently Kirkwood and Bennett (1992) have analyzed the use of regressions. They criticised many published allometric regressions on the grounds that they were rarely accompanied by confidence limits. They further stated that in calculating regressions, X values should be fixed and the Y values variable. Using body weight and BMR, both are subject to inaccuracy. Allometric regressions are influenced by the taxonomic

level at which the analysis is carried out. Due to a disproportionate representation of certain taxa in the analysis or an incorrect classification, large deviations could occur. Kirkwood and Bennett (1992) also showed the large scope for error when an allometric regression is calculated for a group of animals with only a narrow range of body weight (Figure 4).

Even when the correlation coefficient of the regression is large, the 95% confidence limits for the BMR of an animal of average weight (relative to animals used for the calculation) will be 0.5 to 2 times the best estimate. The BMR of the 'average' animal is the most accurate value.

Reasonable estimates can be made for FMR regressions in birds. The slope is about 0.65, values of about 0.60 (4-16, 4-17) being calculated from less reliable data. Differences in intercepts can be explained by differences between breeding and non breeding birds. Most FMR estimations for birds, however, are made for breeding birds, because birds can be recaptured easily returning at their nest.

Consequently allometric regressions could be used to determine the difference in the energy expenditure of birds between laboratory and field. A problem is that confidence limits of the regressions have not been calculated.

Whether a reasonable estimation of allometric FMR regressions can be made for mammals is less evident.

An explanation for this difficulty is that mammal FMRs must be a flexible quantity because of the difficulty of determining the level at which a mammal is active. It can show different levels of activity (for example different types of foraging behaviour), and the energy expenditure will depend on e.g. the temperature of the habitat. Examples of this are antelope FMR that varies, depending on the seasons, from 4 to 6 x BMR. Two species of seals, one much more active than the other, have FMRs of 6 x BMR and 2.7 x BMR respectively. The energy demand of lactating animals is 1.6 times greater than that of non lactating animals.

So FMR regressions will vary in slope and intercept depending on the data used.

When residuals are calculated from allometric regressions ($\log \text{BMR} - \text{expected } \log \text{BMR}$ and $\log \text{FMR} - \log \text{expected FMR}$), animals with a relatively large BMR also seem to have a relatively large FMR. This is a good argument for calculating the FMR/BMR ratio of individual species. Due to better methods of determining FMR, such as the 'doubly labelled water' method, more reliable data will become available in the future. When the purpose is to offer maximum protection to animals, a maximum ratio can be used for quality assessment determinations. The disadvantage of this procedure is the fact that it is difficult to prove that sufficient data from different taxa of birds and mammals are available. From data available now, a maximum ratio of 6 seems reasonable. Calculated from allometric regressions for birds and mammals a ratio of ± 4.5 will be found, at least if significance of the slope of allometric regressions is not considered. Accompanied by confidence limits this

ratio will be 4.5 ± 4.5 , assuming a high BMR always implies a high FMR.

In Appendix B2, the metabolic rates of six individual birds for which BMR, EMR and FMR values are available, are compared. Average FMR/BMR is 4.17 (SD=0.96), average FMR/EMR is 2.68 (SD=0.35).

The energy expenditure of breeding birds approximates the sustained metabolic rate described by Peterson et al. (1990). This FMR/BMR ratio is always lower than 7. Kirkwood and Bennett (1992) considered that maximum energy expenditure was not higher than 6x BMR.

Using SusMR values in quality assessment determinations will offer general protection with larger animals protected more than smaller ones, because they function for less time at a SusMR level.

The maximum energy level determined in the laboratory appears to be about 10 x BMR. Because lactate is produced, this level can not be sustained for a long time.

A risk assessment based on this maximum energy level would sufficiently protect animals even under very demanding circumstances.

Only when an animal uses fat deposits and biomagnification of the compound occurs, it will suffer additional exposure to the compound. This can occur only when the maximal metabolic rate is determined by efficiency of the digestive tract and not by maximal cellular energy metabolism.

In conclusion, an energy level higher than 10 x BMR cannot be sustained for a long period. A SusMR of 6-7 x BMR seems to be a realistic value, and will be lower than a value calculated from allometric regressions with confidence limits. Application of an EMR/BMR ratio of 1.5 to 2, leads to a FMR/EMR ratio of 3 to 4. Using allometric regressions without confidence limits leads to an FMR/EMR ratio of about 2.5. These values do not differ much from the FMR/EMR ratio from Kirkwood and Bennett (1992) and the average FMR/EMR for six individual birds which are 2.5 and 2.68 (SD=0.35) respectively. It is chosen to use values of 2.5 and 4.0 for the ratio's FMR/EMR and SusMR/EMR, respectively. It follows that for the ratio EMR/FMR in equation (2-2) values of 0.4 and 0.25 can be used for average and more extreme energy demanding periods, respectively.

4.3 Caloric content of food items

Toxicity tests with birds and mammals in the laboratory are generally carried out with chickens, quails, mallards, mice and rats. The fodder for these animals is a commercial mixture consisting mainly of grounded cereals and therefore it has a high energetic value. The caloric content of food (CC in equation 2-2) largely determines the amount of food that has to be consumed in order to meet the nutritional demand of the animal. It can be expected that laboratory animals consume less food on a weight basis as compared to raptorial birds and mammals in field conditions predating mainly on small birds and mammals.

A survey of caloric contents of food items for herbivorous, omnivorous and carnivorous birds and mammals is given in Appendix C1. When the caloric content of food items is based on fresh weight a large variation in the caloric content is found: 0.9 to 24.8 kJ/g (see Appendix C1). Differences in caloric content of food items can partly be ascribed to differences in water content; on dry weight basis the variation is

Table 7. Caloric content (CC in kJ/g_{wet weight}) and water content of fodder for laboratory birds and mammals and the important^a food types for raptorial birds and mammals. CF_{bird} and CF_{mammal} are the correction factors for caloric content of the food types for birds and mammals, respectively, calculated by dividing its caloric content by the caloric content of laboratory fodder of birds and mammals, respectively.

Food type	CC mean	CC SD	n	range	%H ₂ O mean	CF _{bird}	CF _{mammal}
Lab. fodder for birds	13.7	2.8	6	11.3 - 17.4	11		
Lab. fodder for mammals	16.8	-	2	11.8 - 22.8	6		
Birds	7.9	2.1	49	3.5 - 12.2	66	0.58	0.47
Mammals	7.1	1.1	19	5.2 - 10.1	71	0.52	0.42
Earthworms	3.0	0.6	3	2.3 - 3.4	84	0.22	0.18
Insect larvae	5.2	3.3	8	1.9 - 11.5	77	0.38	0.31
Insects	7.2	1.6	10	3.2 - 8.8	66	0.53	0.43
Plant leaves ^b	0.9	0.7	7	0.3 - 2.3	92	0.07	0.05
Plant seeds ^b	19.9	6.3	4	16.4 - 29.4	10	1.45	1.18

^a Comprising at least 9% of total diet.

^b This food item is only of importance for one of the mammalian top predators (Badger). The CF_{bird} is therefore not applied in the model calculations.

much smaller; amounting to 15.0 to 28.4 kJ/g. It is chosen to use the fresh weight basis data because BAFs of chemicals in birds and mammals are mostly expressed on fresh weight basis too. The caloric contents of the important food items of the selected top predators are shown in Table 7. The values for the caloric content of birds and mammals are about half of the caloric content values of laboratory fodder for birds and mammals.

4.4 Assimilation efficiency of food items

The assimilation efficiency (FAE in equation 2-2) is the part of the energy present in the food that is assimilated by the organism. The assimilated energy in a diet is the total energy content in a unit of food consumed minus the energy content lost as faeces and urine from the same unit of food. The total energy assimilated by birds and mammals from food is determined by both caloric content and assimilation efficiency which depends on the digestibility. In general less information is available for the assimilation efficiency than for caloric content of food items.

Mammals are somewhat more efficient in the assimilation of energy from their food than birds. When plant leaves, needles and fruits are left out of consideration, the variation in assimilation efficiency of various food types by birds and mammals is relatively small, ranging from 70.5-87.0% and 79.2-89.5%, respectively (Appendix D1, Table 8). In fruit and plant leaves the assimilation efficiency by birds is much lower with approx. 56% and 37%, respectively. The same applies to the food assimilation of plant leaves by mammals. The assimilation efficiency of different food items is comparable with the one from laboratory fodder, which means that the correction factor is approximately one. This leads to the suggestion that a correction for assimilation efficiency will not be necessary for food items like earthworms and insect larvae for which data are lacking. Data for the assimilation efficiency of birds by beasts of prey are lacking but it will probably be about equal to the assimilation efficiency of mammals by beasts of prey. This may be concluded from the assimilation efficiency data of birds of prey, showing that 0.4% more energy is assimilated from birds as food item as compared to mammals as food item. It may be expected that birds and mammals eating mainly plant leaves and fruit have to eat more on a weight basis to reach their nutritional needs, when compared with carnivorous and laboratory animals. This implies that the exposure of xenobiotics in these birds and mammals will probably also be higher. It should be noted, however, that pollutant assimilation efficiency may play a role in this process (see section 4.5).

The correction factor for caloric content in combination with food assimilation efficiency can be seen as a total food correction factor and it increases in the order: bird-eaters, mammal-eaters, invertebrate-eaters.

Table 8A. Food assimilation efficiency (FAE in % of total energy) of different food types by laboratory birds and by raptorial birds. CFbird is the correction factor for the assimilation efficiency of the food item of concern, calculated by dividing its assimilation efficiency by the assimilation efficiency of laboratory fodder.

Food type	FAE mean	FAE SD	n	range	CFbird
Lab. fodder for birds	73.3	9.6	41	42.4 - 92.0	
Birds	74.9	6.8	8	68.0 - 85.5	1.02
Mammals	74.6	7.2	39	61.0 - 94.3	1.02
Earthworms	-	-	0	-	1 ^a
Insect larvae	77.8	5.3	8	70.0 - 86.1	1.06
Insects	67.3	10.1	10	56.0 - 82.7	0.92

^a For earthworms no data were available and an assumption is made for the correction factor (see text).

Table 8B. Food assimilation efficiency (FAE in % of total energy) of different food types by laboratory mammals and by raptorial mammals. CFmammal is the correction factor for the assimilation efficiency of the food item of concern, calculated by dividing its assimilation efficiency by the assimilation efficiency of laboratory fodder.

Food type	FAE mean	FAE SD	n	range	CFmammal
Lab. fodder for mammals	86.1	13.0	3	71.2 - 95	
Birds	-	-	0	-	0.92 ^a
Mammals	79.2	11.2	6	62.7 - 90.1	0.92
Earthworms	-	-	0	-	1 ^a
Insect larvae	-	-	0	-	1 ^a
Insects	85.5	6.5	4	78 - 93	0.99
Plant leaves	49.5	16.8	25	>20 - 89.8	0.57
Plant seeds	87.0	6.1	4	65 - 91	1.01

^a For birds, earthworms and insect larvae no data were available and assumptions are made for the correction factors (see text).

4.5 Pollutant assimilation efficiency

The assimilation efficiency of a pollutant (PAE in equation 2-2) is the amount assimilated divided by the amount consumed. A pollutant has to be absorbed from the alimentary tract into the circulatory system of an organism before it can exert its toxic potential. It is possible that the pollutant assimilation efficiency in laboratory tests may differ from the one of animals in the field.

Several factors have been reported to influence the absorption of pollutants from the gastro-intestinal tract into the blood may be involved:

1. chemical form of the pollutant; free, bound to anions or metallothioneins etc.

Several Cd assimilation efficiency studies have been conducted with laboratory mammals, however not with birds (Appendix E). In a 10 month feeding experiment with rats it is shown that at relatively low Cd concentrations (0.3-3 mg/kg) assimilation efficiency of Cd is comparable for Cd-metlothionein (the form Cd is present in kidney and liver) and CdCl₂ (Groten, 1992). At relatively high Cd concentrations, however, assimilation efficiency of Cd-metlothionein is lower than assimilation efficiency of CdCl₂ (40% lower at 30 mg Cd/kg fodder). Other studies also demonstrated lower assimilation efficiency of Cd in mammals at high Cd concentrations. In plants Cd can be bound to phytochelatin, a protein analogous with metallothionein. The assimilation efficiency of Cd from Cd-phytochelatin seems to be comparable with the one of CdCl₂.

2. binding to the matrix

In natural conditions contaminants are incorporated in organs and tissues, while in laboratory feeding experiments contaminants are often surfacially applied. The chemical will become available for uptake in the gastro-intestinal tract when the matrix to which it is attached is digested. Raptors only partially digest bones, hair and feathers, meaning that pollutants (e.g. Hg, Cd and Pb) in these structures will also be partially made free in the alimentary tract.

The above mentioned study of Groten (1992) demonstrated that Cd incorporated within porcine kidneys fed to rats was equally well absorbed as inorganic Cd surfacially supplied on laboratory food. Clarkson et al. (1984) concluded that methylmercury is assimilated by birds and mammals for 90-100%. Neither binding to the matrix nor chemical form modify the assimilation efficiency of this chemical by mammals (Albanus et al., 1972; Clarkson, 1984).

3. hydrophobicity of pollutant

Only a few experiments have been carried out in which the assimilation efficiencies of lipophilic compounds by mammals and birds are determined. It is assumed that in

natural conditions the assimilation efficiency of the majority of lipophilic compounds by vertebrates is higher than 80% (Moriarty and Walker, 1987). The findings with the absorption of lindane (Oshiba, 1972), HCB (Koss and Koransky, 1975) and PCP (U.S. Public Health Service, 1993) by mammals were in accordance with this. However, the assimilation efficiency of 2,3,7,8-TCDD by birds appeared to be much lower, ranging from 14 to 58%, depending on the type of food (Nosek et al., 1992). Experiments set up to investigate the influence of Kow on assimilation efficiency are not available for birds and mammals. Feeding experiments with fish demonstrated that the Kow of lipophilic compounds (ranging from 4.5 to 8.3) did not systematically influence the assimilation efficiency (50% on average) (Gobas et al., 1993). On the other hand Parkerton (1992) found an inverse relationship between assimilation efficiency and Kow in the freshwater snail *Physa integra*.

4. food quality

The dietary composition may influence absorption processes via proteins, fat and minerals. Moreover digestibility of the food determining the time needed for transport through the alimentary tract may be of importance. For plant leaves this time is generally longer than for plant seeds and animal food types.

Results from experiments with pheasants (Nosek et al., 1992) and starlings (Martin et al., 1989 quoted in: Nosek et al., 1992) suggest that the retention time in the gastrointestinal tract may determine the accumulation of 2,3,7,8-TCDD. The accumulation of this compound was 2-3 times higher when hard-bodied invertebrates served as food as compared to earthworms as food. On the other hand experiments with fish (Sijm et al., 1992) and a freshwater snail (Parkerton, 1992) demonstrated that assimilation of the hydrophobic compounds was inversely related to the digestibility of the food.

Information on the influence of the fat content of food on the assimilation efficiency of lipophilic compounds is absent for birds and contradictory for mammals. In a feeding experiment with rats (Sauberich and Baumann, not dated) mortality induced by DDT was increased by increasing the dietary fat content (0.5%, 5% and 15% fat). In a low protein (10%) diet toxicity was somewhat higher than in a high-protein (30%) diet. Although assimilation efficiency was not measured, DDT toxicity can probably be ascribed to a greater absorption of DDT. On the other hand, benzopyrene absorption by rats was not effected by the fat concentration in the alimentary tract (Lahrer et al., 1984). In fish too, the fat content (<0.2-13.5%) of the food did not systematically effect the assimilation efficiency (Gobas et al., 1993).

5. food quantity

Feeding experiments with a freshwater snail showed that the assimilation efficiency of PCB6 and DEHP was inversely related to dietary ration (Parkerton, 1992) (Appendix E). To our knowledge no attempts have been made yet to investigate the relation

between food quantity and assimilation efficiency of xenobiotics for birds and mammals.

6. species differences

Serafin (1984) found indications for interspecies variation in xenobiotic absorptive ability of birds. The absorption of inorganic mercury and dieldrin in 20 minutes per gram dry weight intestinal segment were higher in the terrestrial species Kestrel, Eastern Screech Owl and Bobwhite Quail as compared to the wetland species Black-crowned night-Heron and Mallard. The authors suggest that this characteristic and the rate of the food passage through the alimentary tract may both be important factors influencing the uptake of xenobiotics in birds.

It can be concluded that for the extrapolation of feeding experiments with mammals in the laboratory to mammals in the field situation corrections for assimilation efficiency of realistic concentrations of Cd and methyl mercury do not have to be made. It is not possible to derive a generally applicable correction for the assimilation efficiencies of organic compounds. The apparent discrepancies in the fragmental data set suggest that species (taxonomical groups) and compound dependent factors may be involved. Therefore $PAE_{lab} = PAE_{field}$ in equation 2-2.

4.6 Species sensitivity

Introduction

Little is known about the actual differences in sensitivity to pollutants between raptorial and non-raptorial birds that might be due to metabolic differences. This so-called *Intrinsic Species Sensitivity* is abbreviated to ISS:

Intrinsic Species Sensitivity : sensitivity dependent on the Xenobiotic Metabolizing System (XMS) of a bird species.

The ISS is a subset of the Species sensitivity (SS) that is defined as:

Species Sensitivity : sensitivity dependent not only on the Xenobiotic Metabolizing System of a bird species, but also on other factors as the type of food, feeding habits, the occurrence of other contaminants in the feed, environmental factors as temperature, other intrinsic factors as sex, moulting and breeding cycles (c.f. Van Straalen and Verkley, 1991).

Many endogenous and exogeneous chemicals are oxidated by the Mixed Function Oxidase (MFO) system. This system exerts a crucial role via catalyzing the oxidation by a "superfamily" of hemoproteins: the cytochromes P₄₅₀ (Sinclair & Sinclair, 1993). The activities of the Mixed Function Oxidase system are considered by many authors as important expressions of the XMS (Walker et al., 1987; Ronis & Walker, 1989; Sinclair & Sinclair, 1993). In this way, differences in the MFO system may reflect differences in sensitivity, or at least to some extent. As the sensitivity of a bird can depend on many factors, it may be concluded that these various components are difficult to disentangle. This was confirmed by Rivière et al. (1985) who compared MFO activities in the Buzzard (*Buteo buteo*) and the Japanese Quail (*Coturnix coturnix*). They stated that it was doubtful to use MFO related enzymatic activities for comparison between species.

Up till now most attempts to quantify MFO differences between and within species have been performed by trapping and killing birds, followed by *in vitro* measurements of enzymatic activities. This *in vitro* approach entails two main drawbacks:

1. To what extent can *in vitro* enzymatic activities be extrapolated to the *in vivo*

conditions of a bird?

2. To what extent contribute *in vivo* differences in the xenobiotic metabolizing rate to sensitivity differences between bird species?

These methodological drawbacks may be resumed as follows: to what extent is the MFO activity in birds a reliable biomarker to predict differences in sensitivity?

Only in a few studies differences in sensitivity within and between species have been compared directly (e.g. Husain et al., 1981; Rivière et al., 1985; Walker et al., 1987). Especially MFO related enzymatic activities in raptorial birds have been scarcely documented.

Ronis and Walker (1989) expressed MFO activity in birds by averaging enzymatic activities with as much MFO substrates (e.g. aldrin, HCE, ethoxy coumarin) as possible.

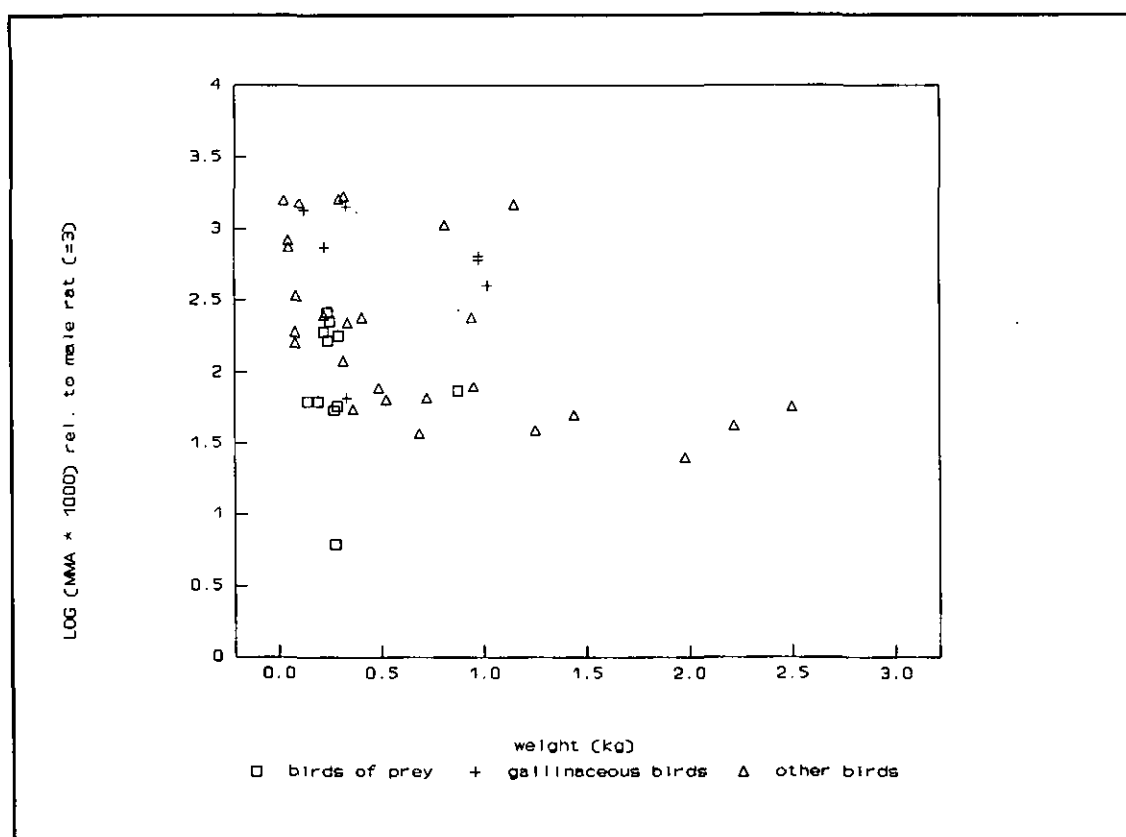


Figure 5. Mean MFO activities (MMA) in individual birds (after Walker et al. (1987), Ronis and Walker (1989)). The single \square , low left in the corner, refers to a juvenile Sparrowhawk.

overlap entirely. In this way comparing the activity towards aldrin in a chicken, with HCE in a Buzzard is comparing apples with oranges.

The second approach assumes all substrates to cause a non-specific reaction of the MFO system. Therefore the activities can be averaged, independent on the type of substrate. This second approach is more or less followed by Ronis and Walker (1989)².

In this subsection the following terms can be distinguished: MA, MMA, MApKG, and MMApKG (see below).

- MA : *
- the MFO Activity in a bird species in comparison with the male rat; per substrate the MFO activity in the male rat is 1;
 - * the MA is per particular type of substrate (e.g. MA_{aldrin});
 - * MA is a dimensionless index;
 - * an MA is calculated as:

$$MA = \frac{\text{activity bird}}{\text{activity male rat}} * \frac{\text{liver weight bird}}{\text{body weight bird}} * \frac{\text{body weight male rat}}{\text{liver weight male rat}} \quad (4-36)$$

- MMA :
- * Mean MFO Activity in a bird species in comparison with the male rat;
 - * the MMA is averaged over all reported substrates per species e.g. MMA^{Kestrel} = [MA_{aldrin} + MA_{HCE}]/2.

The MA includes a correction for liver weight of the bird and is expressed as relative to the male rat (c.f. Walker et al., 1987; Ronis and Walker, 1989). As the MMA is generally negatively correlated with the body weight (see Ronis and Walker, 1989) the MMA is corrected for body weight giving the MApKG per bird species :

² Actually they averaged—if available—the substrates aldrin, aminopyrine, aniline, ethoxy resorufin, HCE, and p-nitroanisole. Ethoxy resorufin was considered as a more specific substrate in comparison with the others.

The results of these *in vitro* experiments are represented in Figure 5¹. This figure suggests that raptorial birds show a lower MFO activity and therefore a higher ISS compared with gallinaceous birds. The hypothesis that different sensitivities for organochlorines occur between raptorial and other birds, and even within raptorial birds has been suggested in various publications (Walker, 1990; Schwarzbach et al., 1991; Newton et al., 1992; Dutch Health Council, 1993; Newton et al., 1993). The main goal of research in this section was to verify this suggestion and to propose a Species Sensitivity Factor (SSF)—if possible—that could be used in extrapolating NOECs of gallinaceous birds to NOECs of raptorial birds. Toxicity data on gallinaceous birds are much more abundant than data on raptorial birds, as the former are commonly used test birds for regulatory purposes. In this way NOECs of raptorial birds could be estimated, merely based on differences in the metabolizing system. The SSF equals $SS_{\text{lab}}/SS_{\text{field}}$ in equation 2-2. Other birds commonly used for testing chemicals as mallard and dove have not been included in chapter 4.6 as the number of *in vitro* data available were considered too small.

Method

Most *in vitro* experiments dealing with differences in biotransformation among bird species have been performed by isolating microsomes containing the MFO system, adding a substrate and subsequently measuring the **potential** enzymatic activity using saturated substrate concentrations.

Then two approaches can be followed:

1. expressing MFO activity in a species for those substrates that were tested in both raptorial and gallinaceous birds (*common* substrates).
2. expressing MFO activity in a species for as much substrates as possible (*common* and *uncommon* substrates).

The first approach avoids confounding variables due to *uncommon* substrates (e.g. the enzymatic activity with aldrin in a Japanese Quail can only be compared with aldrin in a Buzzard, and not with HCE in a Buzzard). This approach assumes rather specific reactions of the MFO system on each type of substrate. In other words, it does not exclude that different subfamilies (i.e. isozymic forms) within the "enzymatic family" of cytochrome P450 may be triggered, or at least that those subfamilies do not

¹ The figures in Figure 5 have been based on the MMAs reported as such in Ronis and Walker (1989), and not on the separate MAs. Unfortunately, the MMAs could not always be verified by simply adding the MAs, as some enzymatic activities were not reported separately, although included in the MMA. E.g., the activity with ethoxy coumarin in the Buzzard was not reported separately. Another point of attention: the authors did not make clear, why for the Buzzard ethoxy coumarin had been included, whereas coumarin (see Rivière et al., 1985) had been excluded.

MApKG : *

- the MFO activity per kg body weight (MApKG of the male rat is always 1); e.g. $\text{MApKG}_{\text{aldrin}}$ for the Barn Owl is 0.09 kg^{-1} ;
- * the dimension of MApKG is kg^{-1} .

MMApKG : *

- Mean MFO Activity per kg b.w.. (e.g. $[\text{MApKG}_{\text{aniline}} + \text{MApKG}_{\text{aminopyrine}} + \text{MApKG}_{\text{aldrin}}]/3$ is $\text{MMApKG}^{\text{Barn Owl}}$);
- * the dimension of MMApKG is kg^{-1} .

The correlation between MMA and body weight is probably due to a relatively smaller food intake of larger birds compared with smaller birds. The latter show larger heat losses in view of a larger surface/weight ratio. It might be expected that small birds show a higher caloric requirement that is correlated with a higher metabolic rate and therefore a higher xenobiotic metabolizing rate. An additional advantage of expressing enzymatic activity as an index is that it meets corrections for differences in the amount of microsomal protein in the liver. In general, activities are represented as e.g. nmol aldrin per mg protein per minute. In stead of expressing the activity in comparison with the rat, one can express the activity as nmol aldrin per animal per minute. The relevant data for this recalculation are often not available. In a study on sea birds however (Knight and Walker, 1982)—including the relevant data—differences between species on the basis of nmol substrate per mg protein per minute were much less pronounced then when expressed as nmol substrate per animal per minute. Examples of the calculation of the MMA, MApKG and MMApKG are presented in Table 9 and 10.

Differences between groups have been tested for significance with single classification analysis of variance (c.f. Sokal and Rohlf, 1969). As there was no homogeneity of variances ANOVAs were carried out with logarithmized MMApKGs.

Results

The most relevant results are resumed in Table 10 and 11. It is important to make clear that all statistical comparisons are based on the enzymatic activities *per species*. The calculations for both aldrin and *all available* substrates have been based on the data in Table 9.

Table 9. Enzymatic activities per substrate per species (MFO activity in the male rat is 1 independent on the substrate). Data derived from Rivière et al. (1985), Walker et al. (1987) and Ronis and Walker (1989).

SUBSTRATE	RAPTORIAL BIRDS			GALLINACEOUS BIRDS			MALE RAT MApKG ^a
	MApKG ^a	SPECIES	N ^b	MApKG ^a	SPECIES	N ^b	
aldrin	0.006 ^h	Buzzard	1	0.02	Pheasant	1	1
	0.05	Sp.hawk ^c	4	0.06	R.l.Partr. ^d	1	
	0.09	Barn Owl	1	0.06	chicken	1	
	0.22	Kestrel	5	0.12	J. Quail ^e	1	
				0.63	Gr.Partr. ^f	1	
aminopyrine ¹	0.06	Barn Owl	1	0.04	chicken	1	1
aniline	0.42	Barn Owl	1	0.34	chicken	2	1
				1.21	Gr.Partr	1	
				0.13	Pheasant	1	
				0.92	J.Quail	1	
ethoxy resorufin	0.01	Buzzard	1	0.56	Gr.Partr	1	1
				0.21	Pheasant	1	
				0.93	J.Quail	1	
				0.46	Bw.Quail ^g	1	
HCE	0.25	Kestrel	5	NDA			1
	0.09	Sp.hawk	4				
p-nitroanisole	NDA			0.32	chicken	1	1
				11.9	Bw.Quail	1	

NDA = no data available. ^a = MFO activity per kg b.w. ^b = number of measurements for determining the (average) activity ^c = Sparrowhawk (*Accipiter nisus*) ^d = Red-legged Partridge (weight assumed to be 0.33 kg; *Alectoris rufa*) ^e = Japanese Quail (*Coturnix coturnix japonica*) ^f = Grey Partridge (weight assumed to be 0.33 kg; *Perdix*) ^g = Bobwhite Quail (*Colinus virginianus*) ^h = A calculation example: the MA in the Buzzard with aldrin as substrate was reported to be 0.017 compared with the rat (MA^{rat} = 1) (see Ronis and Walker, 1989). To obtain the MApKG this value was divided by the weight of the Buzzard (0.874 kg) and by 3.3 (inverse weight of a male rat; via this correction MApKG^{rat} is also 1) ¹ = In Ronis and Walker (1989) two erroneous figures have been reported: the relative activity in the Barn owl with aldrin as substrate should be 0.09 in stead of 0.9 (p.311); the relative activity in the Bobwhite Quail with aminopyrine as substrate should be deleted (reported as 0.067, see p.313).

Table 10. Geometrically mean MFO activities (per kg body weight) for two bird groups.

	Raptorial		Gallinaceous	
	Aldrin	All substrates	Aldrin	All substrates
Mean MFO activity	0.05	0.07	0.09	0.42

Table 11. MFO activity ranges^a (the raptorial vs. gallinaceous birds).

	$\frac{\text{MFO activity raptorial birds}}{\text{MFO activity gallinaceous birds}}$	
Aldrin	0.06 - 5.0	(best estimate 0.56)
All substrates	0.02 - 1.8	(best estimate 0.17)

^a = The ranges of a ratio (raptorial/gallinaceous) have been calculated with the 95% confidence limit of the difference between the logarithmic group (i.e. raptorial vs. gallinaceous) means. The formule is:

$$CL = \bar{x}_1 - \bar{x}_2 \pm t_{0.05[n_1+n_2-2]} \times \sqrt{MSE \left(\frac{1}{n_1} + \frac{1}{n_2} \right)} \quad (4-37)$$

in which:

CL	Confidence limit
Subscript 1	refers to raptorial birds
Subscript 2	refers to gallinaceous birds
MSE	Mean Square Error

Approach of the common substrates

It can be concluded from Table 9 that the substrates tested in both bird groups are aldrin and to a lesser extent aminopyrine, aniline, and ethoxy resorufin. As most data refer to aldrin, focussing on this substrate appears to be most relevant (see Figure 6). In view of the geometrically mean MFO activities (via aldrin) per group (see Table 10) the first group appears c. two times more sensitive than the latter³. This difference is however not significant (ANOVA after logarithmisation, $F_s = 0.46$, $F_{0.05[1,7]} = 5.59$). The lacking of significant differences appears to be due to the small number and the heterogeneity of the data (e.g. the $MAPKG_{aldrin}$ of the Grey Partridge is an

³ In view of the occurrence of high-flyers (see Table 9, e.g. $MAPKG_{Bobwhite\ Quail}$ for p-nitroanisole and $MAPKG_{Grey\ Partridge}$ for aldrin and aniline), the mean is expressed as geometrically.

outlier, see Figure 6). These unsuitabilities of the data also explain why the two bird groups can actually differ largely without being significantly different. This is illustrated in Table 11. The best estimate indicates that raptorial birds are c. two times more sensitive, when focussing on aldrin⁴. Theoretically however, raptorial birds can be 17 times more sensitive than gallinaceous birds⁵.

With respect to the other *common* substrates, it appears that MFO activities between raptorial and gallinaceous birds do not differ, with the possible exception of ethoxy resorufin. The activity with the latter as substrate is in the Buzzard much lower than in the gallinaceous birds (see Table 9).

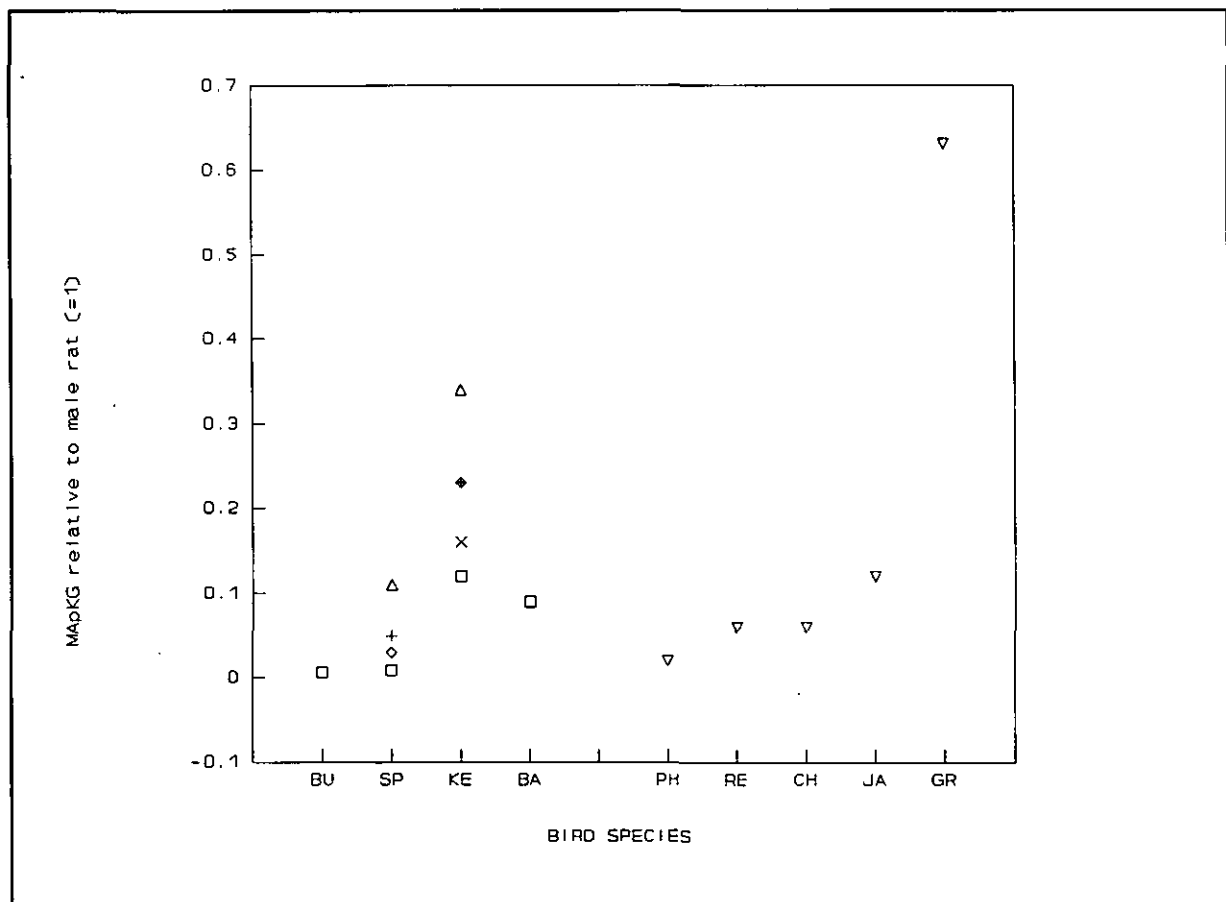


Figure 6. MFO activities (corrected for bodyweight) per individual bird. Activities based on aldrin. BU=Buzzard, SP=Sparrowhawk, KE=Kestrel, BA=Barn owl, PH=Pheasant, RE=Red-legged Partridge, CH=chicken, JA=Japanese Quail, GR=Grey Partridge.

⁴ The reciprocal of 0.56 (see Table 11) is 1.8.

⁵ The reciprocal of 0.06 (see Table 11) is 17.

Approach of the common and uncommon substrates

In view of the geometrically mean MFO activities (via all substrates) per group (see Table 10) the first group appears six times more sensitive than the latter. This difference is however not significant (ANOVA after logarithmization, $F_s = 2.92$, $F_{0.05[1,8]} = 5.32$). The lacking of significant differences may be due to the small number and the heterogeneity of the data (e.g. the MMapKG^{Bobwhite Quail}, based on ethoxy resorufin and p-nitroanisole, is a high-flyer due to the very high activity with p-nitroanisole). These unsuitabilities of the data also explain why the two bird groups can actually differ largely without being significantly different. This is illustrated in Table 11. Theoretically raptorial birds can be 50 times more sensitive than gallinaceous birds, when focussing on all available substrates. The best estimate however indicates that raptorial birds are c. six times more sensitive.

Discussion

From MFO in vitro to XMS in vivo

There are serious drawbacks in extrapolating *in vitro* data on the activities of the MFO system to *in vivo* conditions as has been done in this subsection in order to derive SSFs:

- a. *In vitro* experiments are performed with **saturated** substrate concentrations, indicating an enzymatic potential, rather than an activity under field conditions. Substrate concentrations under field conditions can be much lower. There are however indications of comparable enzymatic activities of raptorial birds under laboratory and field conditions (Walker et al., 1987): at a saturated concentration the mean activity of MFO in two Sparrowhawks was 4% of the activity in male rats, whereas this was less than 5% at much lower concentrations (90 µg aldrin/mg microsomal protein). However, in the latter case, the raw data and the amount of aldrin per mg microsomal protein when saturated were not reported. Conclusively, the assumption that *in vitro* enzyme activities saturated with substrate are comparable with those under *in vivo* conditions and with more realistic concentrations is a weak one and there is almost no scientific evidence for it.
- b. The derivation of SSFs on the basis of the chemical rather than on the species could be misleading as actual differences in accumulating capacities between species may exist. The MMapKGs of Sparrowhawks are relatively low as compared with other raptorial birds (see Table 9). Assuming a high ISS, this might be reflected in various field trials and monitoring studies in which the populations of the Sparrowhawk appear to be affected by very lipophilic organochlorines

more seriously than other raptorial birds (Newton et al., 1993).

- c. It is assumed that the MFO activities are based on non-induced MFO systems. This is however difficult to verify. Induction may lead to new synthesis of enzymes in large quantities whereas the enzyme is normally in a very small amount. The mechanisms for induction are poorly understood so one should be very careful when comparing different species (see also Rivière et al., 1985). It can be expected that when persistent pollutants induce an enzyme this induction will be continued for a long time (pers. comm. Groen, 1993).
- d. Potential differences are amongst others based on the functioning of the MFO system. Although this is supposed to be a very important system in attacking organic pollutants, it definitely does not apply to e.g. heavy metals. It is assumed that the major biotransformation takes place in the hepatic microsomes and that the MFO system is the major enzymatic system in the "phase I" attack. This probably applies to DDT (and metabolites) and dieldrin as their metabolization is rather straightforward. Only this type of lipophilic organochlorines will be metabolized very slowly but largely or entirely by the hepatic MFO system (Walker, 1980). The metabolism of the other organochlorine compounds (lindane and PCP)—for the time being—has to be considered as more complex in a way that the MFO system is only one of the several factors determining the metabolic pathways *in vivo*. Lindane and PCP appear to be less bioaccumulating than dieldrin and DDT (residues). In this way the toxicokinetic and dynamic pattern is expected to be different. For heavy metals it is also difficult to compare differences in sensitivity between species as consistent measurements of the relevant xenobiotic metabolising systems are not available. For some gallinaceous birds high MFO activity has also been found in microsomes in the duodenum (Ronis and Walker, 1989). Husain et al. (1981) found MFO activities in the kidneys of kites (*Milvus migrans*) and vultures (*Gyps bengalensis*) that were comparable to these in the livers. In their *in vitro* experiments a.o. aminopyrine was added to assess the demethylation. In order to understand differences in internal concentrations of pollutants between species one should analyse the uptake and excretion mechanisms of those species (c.f. Van Straalen and Verkley, 1991). However, it appears that apart from the organochlorines DDT (and residues) and dieldrin, toxicokinetic patterns in birds are not well known. A concise overview of some toxicokinetic and toxicodynamic aspects of the pollutants is presented in Appendix F.
- e. It is assumed that the MFO activities do not vary in time. There is however very little known about this aspect. In e.g. the Puffin (*Fratercula arctica*) seasonal fluctuations were demonstrated that were linked with fluctuations in the steroid metabolism during the period of egg laying (Knight and Walker, 1982). Testing the MFO activities of juvenile vs. adult raptorial birds did not

- show significant differences (ANOVA after logarithmization, results not reported here).
- f. It is possible that substantial variation in the MAFKGs (see Table 9) is due to different methods of preparing and conserving microsomes (personal comm. Groen, 1993). The low activities in the Buzzard with aldrin and ethoxy resorufin (see Table 9) may be due to the low temperature during the assays (30°C). The higher activities in the Kestrel and the Sparrowhawk with aldrin were assayed at 42°C).
 - g. The MFO activities in this section are regarded as the most relevant expressions of the XMS. They are considered as the transformation rate limiting factor. This may be appropriate for highly lipophilic chemicals, whereas this is doubtful when "phase II" enzymes or other enzymes are included (e.g. the enzymes related with glutathione conjugation may be very important in the detoxification of many compounds (Mennes, 1992)).
 - h. Structural differences in MFO activities with respect to various substrates might be explained by the existence of different "subfamilies" (isozymic forms) within the P₄₅₀ "superfamily" with different and overlapping substrate specificity (Rivière et al., 1985). Data on the occurrence of different subfamilies in birds are limited: the subfamily IA was found in the Cormorant and the Bobwhite Quail, whereas the subfamily IIC was only found in chicken (Leonzio et al., 1992; Nebert and Gonzales, 1987; Walker et al., 1992). Therefore it is actually not possible to agree on the best approach of comparing enzymatic activities (via *common* or via *all available* substrates⁶).
 - i. The term Intrinsic Species Sensitivity is confined to toxicokinetic aspects of a chemical. For reasons of convenience possible differences in the toxicodynamic aspects, as e.g. in the receptor sensitivities, are not taken into account.

Raptorial versus gallinaceous birds

In view of the various methodological drawbacks, the MFO activity does not appear to be a reliable biomarker in order to explain the possible differences in SS between gallinaceous and raptorial birds. This is conform the conclusion of the Dutch Health Council (1993) that in view of the large variability of laboratory data no reliable predictions can be made for the sensitivity of top predators in the field. The results of the statistical tests however do not support the conclusion of the Dutch Health Council that it was proven that MFO activities in raptorial birds are lower than in

⁶ It could be stated tentively that the "truth" is somewhere between: most substrates, "offered" to birds in the field, trigger a group of isozymes (so not a very particular isozyme on one hand and not all isozymic forms on the other hand).

non-raptorial birds⁷.

To what extent could differences in MFO activity be extrapolated with respect to other chemicals? A complicating factor is that an increased MFO activity may lead to a higher extent of (potential) detoxification of some chemicals, whereas it may enhance the toxicity of others. An example had been reported by Johnston et al. (1990) in which the fungicide prochloraz strongly induces MFO activity in chickens. As via MFO more malathion was transformed to the more toxic malaoxon rather than to malathion monoacid, the birds became more susceptible to this pesticide. Conclusively, it can be stated that only detailed knowledge especially about the toxicokinetic aspects of chemicals could be decisive in determining an SSF on the basis of the chemicals mentioned here.

Owls (Strigiformes) are considered as raptorial birds. Differences in the toxicokinetic and dynamic patterns with respect to the Falconiformes however cannot be excluded as application of a PCB mixture had been reported to induce MFO activity in the Barn Owl and the Japanese Quail, but not in the Buzzard (Rivière et al., 1985).

The actual existence of differences between the two bird groups is possibly supported by the striking difference between control Buzzards and Japanese Quails, when testing 7-ethoxycoumarin (Rivière et al., 1985). This substrate was metabolized much faster by the quails. Other indications for actual differences in SS between bird groups have been reported by Newton (1979): comparable concentrations in eggs may result in different eggshell thinning percentages. Concentrations of 4 - 5 mg DDE/kg in fresh eggs of raptorial birds were correlated with an eggshell thinning $\geq 15\%$. Comparable concentrations in the eggs of song birds and wild fowl resulted in eggshell thinning of 10% and 1%, respectively. This difference in SS however could also be due to different diets, habitat or simply a higher feeding rate. Greater internal exposure could be simply due to e.g. a higher feed intake or the consumption of more contaminated animals. Also the capability of some birds of prey to digest bones could cause a greater internal exposure of e.g. lead.

Differences in ISSs between birds could speculatively be due to different induction responses. This may be explained with the following example in which Buzzards had been compared with Bobwhite Quails. In an *in vitro* experiment, the effects of a commercial PCB mixture on MFO activities in the livers of the aforementioned bird species were investigated (Rivière et al., 1985). The MFO activity in the Bobwhite Quail in general increased after application of the PCBs whereas there was no response in the Buzzard. The enzyme activity increase in the Bobwhite Quail was at

⁷ It is stated in some sources that seabirds may be more sensitive to chemicals than gallinaceous birds. However, a comparable statistical practice as performed for the raptorial and gallinaceous birds shows no significant differences between the gallinaceous birds and seabirds (data from Ronis and Walker, 1989; results not published here). So far as differences occur, these are less pronounced than between gallinaceous birds and raptorial birds.

most 2 times the control (NADPH-cytochrome *c* reductase). The activities in the control Buzzards and Bobwhite Quails were comparable. The different induction responses were possibly reflected in the concentrations in the adipose tissue of the PCB-treated birds: 78 ± 52 mg/kg b.w. (Buzzard, $n = 4$), 28 ± 9 mg/kg b.w. (Bobwhite Quail, $n = 3$). This difference however could also be caused by different activities of "phase II" enzymes as the activity of glutathione S-transferase in the Bobwhite Quail was much higher in both the treated and untreated group compared with the Buzzard. Non-inducement was also shown in Cormorants after application of dieldrin or phenobarbital, whereas these chemicals can induce the MFO system in e.g. domestic fowl and Japanese Quail (Ronis and Walker, 1989; Walker and Ronis, 1989).

Only a few data on the activities of the MFO system between raptorial and gallinaceous birds are available. Two approaches have been used to quantify the XMS activities in these groups: the first via aldrin, the only substrate tested in both groups, the second via all available substrates that were tested in either raptorial or gallinaceous birds. This is simply because it cannot be judged whether a certain substrate should be able—*in extemo*—to trigger all isozymic forms of a P_{450} cytochrome or rather one. Nevertheless it may be expected that comparison of *in vitro* tests with the same substrate will give better opportunities for comparison. In this way it may not be surprising that the differences in the MFO activities between the two bird groups were much smaller when based on aldrin than when based on as much substrates as possible.

Particularly when comparing raptorial with gallinaceous birds on the basis of *in vitro* tests, in Walker et al. (1987) and Ronis and Walker (1989), with *all available* substrates there is a simple methodological pitfall: some *uncommon* substrates show high activities that have been included in the MMapKG (e.g. p-nitroanisole in gallinaceous birds), whereas no comparable values in raptorial were available. Therefore the MMapKG is much dependent on the accidental availability of data.

As noted before, despite the lack of statistical differences, actual differences between groups or species cannot be excluded. But even when systematic statistical differences *for some substrates* should be found, one should avoid to jump to conclusions, as for other substrates the pattern can be just the opposite. The main reason is the complexity of the XMS. Very slight differences in the structure of a hemoprotein may lead to interactions with completely different substrates. E.g. a rat can be considered closely related with a hamster (mammals of comparable size), whereas the *in vitro* results with various substrates differ substantially (pers. communication Mennes)⁸. As

⁸ Probably the same methodological problems arises when comparing common test mammals (e.g. rat, hamster) with ecologically important raptorial mammals as e.g. the Badger and the Weasel. Almost no data on the MFO activities in these or other raptorial mammals are available, although the literature search has not been exhaustively. Walker (1980) reported for the Cat (*Felis catus*) an MApKG_{aminopyrine} of 0.06 kg^{-1} and an

these species were tested under comparable conditions (e.g. feed), it implies that even closely related species may differ substantially.

Conclusions

No significant differences in MFO activities between the two bird groups have been found, in spite of the "extreme" approaches (i.e. *common* substrates vs. *all available* substrates). Therefore, an SSF of 1 in order to extrapolate NOECs of gallinaceous birds to NOECs of raptorial birds—with respect to MFO intermediated chemicals—is scientifically sound. However, it cannot be excluded that differences in MFO activities actually exist, as theoretically the activities in raptorial birds may be 17 times lower than those in gallinaceous birds, without being significantly lower. It does not seem appropriate to use this extreme value for establishing an SSF, because of the few available data and the lack of knowledge whether a specific substrate triggers only one isozymic form, rather than—in *extremo*—all isozymic forms.

The approach of Ronis and Walker (1989), who used (almost) all available data on enzymatic activities related to the MFO system, is not appropriate for comparing raptorial with gallinaceous birds. Not only because of the drawbacks abovementioned, but also because of the "lack of balance" in the data available (e.g. activities with p-nitroanisole are only available for gallinaceous birds).

It cannot be excluded that a "refined" set of *more* data will reveal significant differences. This can lead to the conclusion that for a *particular* chemical e.g. a lower MFO activity corresponds with a lower probability of accumulation⁹. However, such conclusions cannot be drawn yet. Tentatively, it could be stated that the (eco)toxicological meaning of *in vitro* measurements of the MFO system is limited, unless more really comparable measurements become available. These physiological measurements as a measure for ISS can be compared with toxicity tests (e.g. LC₅₀) as the latter may reflect differences in the sensitivity that are dependent on more factors than the XMS alone¹⁰.

Conclusively it should be noted that with the current data-set it is difficult to assign differences in the Xenobiotic Metabolizing System—if any—to different bird groups, let alone bird species. Difficulties in determining differences between mammalian species appear to be documented much better (see Smith, 1987; Gilette, 1989; Tsyrov and Duzchak, 1990), and may serve therefore as a guideline for generating more useful

M_{Ap}K_G_{aniline} of 0.10 kg⁻¹. Therefore the M_{Map}K_G^{Cat} is 0.02 kg⁻¹. This appears to be much lower than in the male rat.

⁹ This may be appropriate for the very lipophilic compounds, for which the oxidation via P₄₅₀ is considered as limiting.

¹⁰ An example of such an approach was presented in Walker (1983)

data for comparing birds. It appears especially from Gillette (1989) that one should be very alert in extrapolating the activities for *some* to *all* substrates.

5. AVAILABILITY OF BIOACCUMULATION DATA

A survey of the availability of BCFs and BAFs of the six selected chemicals for each group is given in Table 14. These values are used for the construction of the terrestrial food webs and are compiled from the studies with bioaccumulation data shown in the Appendices H up to O. For the selected organic compounds, BCFs of the invertebrates are lumped in two separate groups: soft-bodied and hard-bodied invertebrates. Furthermore BCFs for leaves are applied for fruits too. For methyl mercury, BCFs for earthworms and leaves are used for all invertebrates and seeds, respectively. The geometric mean BCFs and BAFs for the selected organic compounds and for methyl mercury in the simplified food webs are listed in Appendix H4 and H5, respectively.

Table 14. A survey of BCFs and BAFs ($[\text{kg}_{\text{dry soil}}/\text{kg}_{\text{wet tissue}}]$, geometric means) of selected chemicals in plant parts, invertebrates, birds and mammals. The BAFs are whole body BAFs. For Cd, lindane and PCP in birds and for lindane, methyl Hg and Cd in mammals whole body BAFs are calculated from BAFs in target organs and tissues.

	BCF, BAF					
	DDT	dieldrin	lindane	PCP	MeHg	Cd
Leaves	0.05	0.04	0.15	0.82	0.06	0.07
Fruits					0.01	0.01
Seeds	1.02		2.71	0.05		0.46
Tubers	0.002	0.06	0.86		0.01	0.05
Earthworms	0.17	0.36		1.51	8.28	3.74
Gastropods	0.68	0.32		0.002		1.67
Insect larvae	0.46					0.85
Caterpillars						0.25
Insects	0.63	0.59	0.64	0.06		0.54
Isopods	2.76			0.03		7.37
Spiders			0.03	0.13		6.05
Birds	4.25	1.18	0.098	0.014	0.94	0.053
Mammals	1.42	0.63	0.106	0.080	3.39	0.034

In birds and mammals, BAFs of the chemicals in the target organs and tissues and the calculation of a BCF on whole body basis from these BCFs are shown in Table 15, respectively.

The available BCFs and BAFs are discussed below for each chemical separately.

Table 15. BAFs ($[\text{kg}_{\text{dry soil}}/\text{kg}_{\text{wet tissue}}]$, geometric means) of six selected chemicals in target organs and tissues of laboratory birds (A) and laboratory mammals (B). A whole body BAF is calculated with these BCFs and the relative weights shown in Appendix O1. Available experimental whole body BAFs are also shown.

A: Birds

	DDT	dieldrin	lindane	PCP	MeHg	Cd
fat	5.61	12.46	1.03	0.06		
kidney		60.32	0.05	0.12	2.80	2.72
liver	1.86	0.62	0.02	0.07	2.82	0.92
muscle		0.30	0.01	0.02	1.04	0.04
body (exp.)	4.25	1.18			0.94	
body (calc.)	0.54	1.22	0.097	0.014	0.40	0.053

B: Mammals

	DDT	dieldrin	lindane	PCP	MeHg	Cd
fat	6.22	3.80	0.64	0.05		
kidney			0.27		12.00	0.74
liver	0.11	0.15	0.00	0.24	6.67	0.26
muscle			0.08		5.50	0.02
body (exp.)	1.23	0.63		0.08		
body (calc.)	0.59	0.36	0.106	0.017	3.39	0.034

DDT

DDT is hardly accumulated from the soil by the vegetation. However, accumulation of DDT by plant seeds is much higher, although this is based on only one study. There are several DDT bioaccumulation studies with invertebrates, however the spectrum of species is limited. With the exception of isopods, BCFs are generally below one.

DDT-BAFs of vertebrates are mostly reported on lipid basis. However, the body lipid percentage is highly variable from 0.5 to 35 for both birds and mammals (Appendix O1) making it difficult to extrapolate these data to a BAF on body basis. In starving birds fat reserves rapidly decrease leading to increased DDT concentrations. It is preferred to use the few DDT-BCFs on body basis because total lipid content of bodies are often not reported. This may also explain the large difference between the experimental DDT-BAFs on body basis and the DDT-BAFs calculated from bioaccumulation in fat of both birds and mammals. Biomagnification is higher in birds than in mammals.

The highest BAFs in fat are found for birds of prey, followed by small birds, mammals and beasts of prey. So it seems that the most critical routes for exposure to DDT are: *invertebrates/seeds* → *small bird* → *bird of prey*.

Much higher DDT-BAFs are found in laboratory experiments carried out with raptorial Barn Owls and American Kestrels (*Falco sparverius*), and the fish-eating Herring Gulls (*Larus argentatus*), White Pelican (*Pelecanus erythrorhynchos*) and Double-crested Cormorant (*Phalacrocorax a. auritus*) (Appendix I5).

Dieldrin

Like with DDT bioaccumulation of dieldrin is relatively low in plant leaves and tubers.

Studies on the accumulation of dieldrin have been carried out for earthworms, beetles and gastropods. In these invertebrates biomagnification of dieldrin is absent.

In vertebrates biomagnification of dieldrin is more often expressed on body lipid basis, than on whole body basis (Table 15). Accumulation in birds is about twice the accumulation in mammals. Dieldrin BCFs and BAFs used in the model have values around or below one. It is however possible that biomagnification of dieldrin occurs with either raptorial birds or birds in general under natural conditions. Indications for this come from laboratory experiments with Barn Owls and Prairie Falcons (*Falco mexicanus*) as well as from field data concerning fish-eating Herring Gulls and Shags (*Phalacrocorax aristotelis*) (Appendix J5).

Lindane

Biomagnification (BCFs > 1) occurs in plant seeds but not in plant leaves and tubers. It should be noted that the data sets with BCFs of organic chemicals comprise only agricultural crop species. BCFs of lindane for plants are higher than the BCFs DDT and dieldrin. This is in accordance with Wild and Jones (1992) who reported the highest BCFs for organic compounds with intermediate K_{ow} s.

Information on bioaccumulation of lindane in invertebrates is scarce. Among the

invertebrate species a large variation exists in BCFs, although these BCFs are generally below one. This is true for both BCFs based on concentrations in soil and for BCFs based on concentrations in food (Appendix K2).

Lindane does not biomagnify in birds and mammals on a whole body basis. This can be concluded from the experimental data available for the accumulation of lindane in fat of Chickens and Quails and rats (Table 15, Appendices K3 and K4).

PCP

Experimental data on the accumulation of PCP in plants are scarce. For plant leaves and seeds mean BCF appear to be below 1. However, details about plant species and soil parameters etc. are not reported.

For the most invertebrate groups PCP-BCFs could be collected. However the number of determinations for each species is very low. Biomagnification was observed for earthworms only. BCFs for the other invertebrates range from 0.002 to 0.18. In soft-bodied invertebrates BCFs are higher than in hard-bodied invertebrates.

PCP-BAFs for birds and mammals are based on two experiments with chickens and one experiment with a Prairie Vole, respectively. It is clear that bioaccumulation of PCP in fat of these animals is low (Table 15, Appendices L3 and L4).

Methyl Hg

Bioaccumulation of MeHg (Appendix M1) in plants is based on one detailed study (Cappon, 1987) in which a distinction is made between methyl Hg and inorganic Hg in both soil and plants. Methyl mercury BCFs in leaves, fruits and tubers appear to be negligible, although still four times higher than the inorganic mercury BCF in these plant parts.

There is only one study available in which MeHg concentrations were determined in both invertebrate tissue and soil. BCFs based on concentrations in food are available for carnivorous insect larvae, carnivorous insects and herbivorous insects. The average BCFs for insect larvae and adult insects amount to 3.82 and 2.56, respectively, but are not applied in the model calculations. The BCF for earthworms is used for both soft-bodied and hard-bodied invertebrates.

For birds a large data set of whole body BAFs and organ BAFs is available. BAFs in kidney and muscle are about a factor three higher than BAFs of body and muscles. For the regular laboratory mammals BAFs are only available for kidneys. Determinations of MeHg in other organs and tissues have been carried out with fur-bearing mammals. A whole body BAF of 3.4 is calculated from these BAFs in target organs and tissues. MeHg strongly accumulates in hair and feathers, but it is not available for uptake by raptors, because hair and feathers are not digested by raptors.

Inorganic Hg

There are very few studies from which accumulation of inorganic Hg can be calculated (Appendix M7). This concerns two invertebrate species, one avian species and one mammalian species. Biomagnification is reported only for larvae of blowflies (*Lucilia illustris*) and in the kidney of the Mink (*Mustela vison*). This implies that a complete picture of food web transfer can not be obtained. The risk of secondary poisoning by inorganic Hg is negligible because these BCFs and BAFs are very low. However, there will be a risk in case a substantial part of inorganic Hg in the soil is converted into methyl Hg.

Cadmium

For plants Cd BCFs are below 1. Cadmium-BCFs in plant seeds are substantially higher than in the other parts. A large variation exists in BCFs of a plant part and this may be influenced by several factors: Cd concentration, soil-pH, soil-CEC and plant species and in some studies relations of these factors with the Cd concentrations in the plant are reported (Appendix N1).

A large data set on Cd-BCFs for invertebrates is available, concerning many species. The variation between these species in Cd-BCF is high: ranging from 0.25 to 7.44. Biomagnification (BCF value higher than one) is found for earthworms, gastropods, spiders and isopods. The lowest BCFs are found for insects, both larvae and adults. In many studies Cd concentrations in the litter layer are also measured. These Cd concentrations are generally higher than the Cd concentrations in the soil which means that Cd-BCFs based on Cd concentrations in the litter are lower than the BCFs based on concentrations in the soil (Appendix H8).

Accumulation of Cd in the target organs of birds and mammals has been extensively studied (Appendices N3-N7, Table 15). However, the number of species is limited. In birds a clear biomagnification occurs in the kidney. In the liver a BCF of one is found, whereas in muscles Cd accumulation seems to be absent. In laboratory mammals biomagnification of Cd does not take place. Even in kidneys BAFs are generally below one. Cd-BAFs in kidney are about three times the Cd-BAFs of liver in both birds and mammals. A Cd-BAF for whole body is estimated by multiplying the Cd-BAFs in kidney, liver and muscles with their relative organ/tissue weights (Appendix H6). The calculated Cd-BAFs for whole body are low for both birds and mammals.

It should be considered that the exposure period is important when studying Cd accumulation. The average exposure periods for kidney, liver and muscle amount to 17, 17, and 22 weeks, respectively for birds and 24, 22, and 7 weeks, respectively for mammals.

6. CALCULATIONS

6.1 Toxicity data corrected for top predators

The NOECs of the selected compounds in birds and mammals in Romijn et al. (1991b) are extended with an update (Table 16, 17). Equation (6-1) is used to correct each laboratory bird NOEC to a NOEC for birds of prey under natural conditions. The relevant correction factors for two diverse types: bird-eater and mammal-eater and the selected species are listed in Table 18. The same procedure is followed for the correction of laboratory mammal NOECs to beast of prey NOECs. That implies that corrections have to be made for metabolic rate (0.4), food caloric content and food assimilation efficiency. In case information on these items is not given in these studies, it is assumed that it is most appropriate to use an average caloric content and assimilation efficiency as shown in the Table 7 and 8, respectively.

$$NOEC_{speciesofconcern} = NOEC_{lab} * \frac{EMR}{FMR} * \frac{CC_{field}}{CC_{lab}} * \frac{FAE_{field}}{FAE_{lab}} \quad (6-1)$$

The proportion of birds and mammals in the diet causes differences in the food corrections for caloric content and assimilation efficiency, with a higher correction for the typical mammal-eaters (e.g. Kestrel), as compared with the typical bird-eaters (e.g. Sparrowhawk). According to this method the highest correction factor should be applied to the Little Owl and the Badger due to the consumption of invertebrates like earthworms. The total correction among the selected species ranges from 0.17 to 0.24 for raptorial birds and from 0.15 to 0.16 for raptorial mammals (Table 18).

There are few laboratory toxicity tests carried out with raptorial species. After correction, DDT-NOECs for American Kestrel (*Falco sparverius*) and Eastern Screech Owl (*Otus asio*) and the MeHg NOEC of Mink (*Mustela vison*) are within the range of the corrected NOECs of other birds and mammals, respectively. On the other hand, the corrected methyl Hg NOEC of the Red-tailed Hawk (*Buteo jamaicensis*) is somewhat higher than the ones of other birds (Appendix G1). It suggests that the sensitivity of raptorial and other species are comparable.

It should be noted that toxicological data for effects of PCP and lindane on birds are extremely scarce. Therefore NOECs of these compounds can not be treated as stochasts in the calculations of MPCs and the modified EPA method has to be applied for deriving a NOEC for birds and a NOEC for mammals.

Table 16. Dietary toxicity data (NOECs) of selected chemicals for birds in laboratory experiments. After application of the correction factors (Table 18) these data are used for the calculation of MPCs.

Chemical	Parameter	Species	NOEC (mg/kg food)
DDT (total)	re	<i>Streptopelia risoria</i>	0.50
	re	<i>Gallus domesticus</i>	0.60
	mo	<i>Molothrus ater</i>	3.3
	re	<i>Anas platyrhynchos</i>	3.3
	re	<i>Coturnix c. japonica</i>	10
	mo	<i>Colinus virginianus</i>	17
	re	<i>Phasianus colchicus</i>	50
	re	<i>Falco sparverius</i>	5.6
	re	<i>Otus asio</i>	2.8
Dieldrin	mo	Quail sp.	0.50
	re	<i>Anas platyrhynchos</i>	0.80
	mo	<i>Numida meleagris</i>	1.5
	re	<i>Phasianus colchicus</i>	2.0
	mo	<i>Colinus virginianus</i>	2.5
	re	<i>Gallus domesticus</i>	10
	mo, re	<i>Coturnix c. japonica</i>	10
lindane	mo	<i>Gallus domesticus</i>	1.6 *
PCP	re	<i>Gallus domesticus</i>	100 *
Methyl Hg	re	<i>Anas platyrhynchos</i>	0.25
	mo, gr	<i>Phasianus colchicus</i>	0.36
	mo	<i>Gallus domesticus</i>	0.56
	mo	<i>Coturnix c. japonica</i>	1.7
	mo	<i>Colinus virginianus</i>	4.3
	mo	<i>Poephila guttata</i>	2.9
	mo, gr	<i>Buteo jamaicensis</i>	3.9
Cd	gr	<i>Meleagris gallopavo</i>	0.20
	re	<i>Anas platyrhynchos</i>	1.60
	mo, re	<i>Gallus domesticus</i>	12
	gr	<i>Coturnix c. japonica</i>	38
	re	<i>Streptopelia risoria</i>	1.9

* A safety factor of 10 has to be applied to this value according to the modified EPA method
mo = mortality, re = reproduction, gr = growth

Table 17. Dietary toxicity data (NOECs) of selected chemicals for mammals in laboratory experiments. After application of the correction factors (Table 18) these data are used for the calculation of MPCs.

Chemical	Parameter	Species	NOEC (mg/kg food)
DDT (total)	re	Rattus norvegicus	20
	re	Mus musculus	25
	mo	Saimura sciureus	28
	mo	Microtus pennsylvanicus	100
	mo,gr	Macaca mulatta	200
	mo	Canis domesticus	400
Dieldrin	mo	Mus musculus	1
	re	Macaca mulatta	1
	re	Rattus norvegicus	1.25
	mo	Blerina brevicaudus	5
	gr	Canis domesticus	8
	mo	Damaliscus dorcas p.	15
lindane	re	Mus musculus	25 *
PCP	re	Rattus norvegicus	55 *
Methyl Hg	gr	Macaca sp.	0.22
	gr,re	Rattus norvegicus	0.43
	mo	Mustela vison	1.20
	gr	Mus musculus	2.25
Cd	gr	Macaca mulatta	3
	gr	Ovis amon aries	15
	gr	Rattus norvegicus	20
	gr	Bos primigenius taurus	40
	gr	Sus scrofa domesticus	45

* A safety factor of 10 has to be applied to this value according to the modified EPA method
 mo = mortality, re = reproduction, gr = growth

Table 18. Survey of correction factors applied for the correction of standard laboratory NOECs to NOECs for four different top predator types (A) and the selected top predator species (B).

A: Top predator types

Type	Correction factors			Total
	Caloric content of food	Food assimilation efficiency	Metabolic rate	
<u>Birds of prey</u>				
Bird-eater	0.58	1.02	0.4	0.24
Mammal-eater	0.52	1.02	0.4	0.21
<u>Beast of prey</u>				
Bird-eater	0.47	0.92	0.4	0.17
Mammal-eater	0.42	0.92	0.4	0.15

B: Selected species

	Correction factors			
Species	Caloric content of food	Food assimilation efficiency	Metabolic rate	Total
<u>Birds of prey</u>				
Sparrowhawk	0.58	1.02	0.4	0.24
Goshawk	0.57	1.02	0.4	0.23
Buzzard	0.53	1.02	0.4	0.22
Kestrel	0.52	1.02	0.4	0.21
Long-eared Owl	0.53	1.02	0.4	0.22
Tawny Owl	0.49	0.93	0.4	0.18
Barn Owl	0.52	1.02	0.4	0.21
Little Owl	0.43	0.99	0.4	0.17
<u>Beasts of prey</u>				
Weasel	0.44	0.92	0.4	0.16
Badger	0.42	0.87	0.4	0.15

6.2 Accumulation in food webs of top predators and soil quality criteria

For the selected chemicals most BCFs among the first trophic level components are in general available for leaves, seeds, worms and insects. Therefore it is decided to pay special attention to eight separate food chains comprising one of these components, birds or mammals at the second trophic level and raptorial bird or mammal at the third trophic level. In addition, MPCs will be derived on basis of the bioaccumulation through the eight separate food chains.

The distributions of the total bioaccumulation of the selected chemicals from the soil through single food chains and as well as into the food of the ten top predators species are calculated. The median BAF (BAF_{50}) values are presented in Table 19. The 25th (BAF_{25}), 50th (BAF_{50}) and 75th percentiles (BAF_{75}) of the BAF distributions for the selected species are listed in Appendix H7.

MPC distributions are generated from distributions of corrected NOECs and BAFs. Special attention will be paid to the MPC_5 , representing the soil concentration with a probability of 5% that NOECs will be exceeded (Table 20). The 5th (MPC_5), 25th (MPC_{25}), 50th (MPC_{50}) and 75th percentiles (MPC_{75}) of these distributions for the selected species are also shown in Appendix H7.

The variation in BAFs and in MPCs among food chains and selected species will be discussed for each chemical. Exposure through the single food chains will indicate minimum and maximum MPCs that may be expected for top predators. It should be kept in mind that there are very few terrestrial top predator species which are exposed through one food chain. The Kestrel is the only example among the selected species being exposed almost entirely (95%) through one food chain, namely *soil* → *plant leaf* → *mammal*.

6.2.1 DDT

Food chains

The bioaccumulation of DDT from soil is the highest in seed-eating and insect-eating birds and mammals and the lowest in leaf-eating birds and mammals. The BAF_{50} for DDT ranges from 0.067 for leaf-eating mammals to 4.37 for seed-eating birds. It should be noted that the variation in BAF among insect-eaters is much higher than among the seed-eaters.

Exposure through the food chain *soil* → *insect* → *bird* leads to the lowest MPC_5 s with values of 11 and 30 $\mu\text{g}/\text{kg}$ for avian and mammalian top predators, respectively, closely followed by subsequently the food chain *soil* → *seed* → *bird*, *soil* → *insect* → *mammal* and *soil* → *seed* → *mammal*. The highest MPC_5 s are found for avian and mammalian top predators consuming leaf-eating mammals being about a factor 50

higher than for top predators consuming only insect-eating birds.

Selected species

The exposure to DDT varies considerably both among and within the selected birds as is illustrated by figure 6. The calculated DDT-BAF₅₀s range from 0.16 in the Kestrel to 3.34 in the Sparrowhawk (Table 19). The bird-eating Sparrowhawk and Goshawk are the only species with a median BAF₅₀ higher than one. The exposure to DDT in the food is lowest for mainly herbivorous mammal-eating top predators like Kestrel and Long-eared Owl, with BAF₇₅ values below one.

The DDT-BAF₅₀ for the Badger is slightly higher than the one for the Weasel, amounting to 0.63 and 0.42, respectively.

Figure 7 provides a view of the variation in DDT-MPCs both within and among species. In general, MPCs increase in the order: bird-eaters (Sparrowhawk, Goshawk), invertebrate-eaters (Little Owl), carnivorous mammal-eaters (Barn Owl, Buzzard), herbivorous mammal-eaters (Kestrel, Long-eared Owl). The Sparrowhawk is the most critical species for secondary poisoning by DDT with a MPC₅ of 15 µg/kg soil. This MPC₅ is about a factor 13 lower than the highest MPC₅ among the selected raptorial birds (Kestrel).

The MPC₅ for the Badger (75 µg/kg) is about half the MPC₅ for the Weasel. The MPC₅s of the selected birds are all lower than the MPC₅s for the selected mammals. This can be ascribed to the fact that mammals are far less sensitive to DDT than birds.

The estimated sensitivity, exposure, and risk of secondary poisoning of the Sparrowhawk are illustrated by the frequency distributions of corrected NOECs, BAFs in its food and MPCs in the soil, respectively (Figure 8). The MPC distribution can be described by a log-logistic function, which can be seen when a box plot is made on a log scale (Figure 7B).

6.2.2 Dieldrin

Food chains

The bioaccumulation of dieldrin is the highest for the food chain *soil → insect → bird*, followed by *soil → worm → bird*, *soil → insect → mammal*, *soil → worm → mammal*, *soil → leaf → bird*, and *soil → leaf → mammal*. For seed-eaters no bioaccumulation can be calculated due to the absence of experimental data for bioaccumulation in seeds. The accumulation by insect-eating birds (0.70) is a factor 28 higher than by leaf-eating mammals (0.025).

MPC₅s for dieldrin on basis of the six food chains range from 36 to 750 µg/kg for

birds of prey and from 38 to 670 $\mu\text{g/kg}$ for beast of prey with the lowest MPC₅s for exposure by the food chain insect-bird and the highest values for the food chain *soil* \rightarrow *leaf* \rightarrow *mammal*. The MPC₅s for top predators consuming worm-eaters are twice the MPC₅s for top predators consuming insect-eaters.

Selected species

The calculated dieldrin-BAF₅₀s from soil to food are below 0.31 for all species. The variation in these BAF₅₀s among the selected raptorial birds the food is relatively small, ranging from 0.031 for the Kestrel to 0.31 for the Sparrowhawk. BAF₅₀ values decrease in the order: bird-eaters (Sparrowhawk and Goshawk), invertebrate-eaters (Little Owl), species eating relatively many insectivorous mammals (Barn Owl, Buzzard), herbivorous mammal-eaters (Kestrel, Long-eared Owl).

The same phenomenon is observed for the mammalian raptors with a higher BAF₅₀ for the Badger (0.26) than for the Weasel (0.057).

The MPC₅ of dieldrin for raptorial birds ranges from 78 $\mu\text{g/kg}$ for the Sparrowhawk to 600 $\mu\text{g/kg}$ for the Long-eared Owl. MPC₅s for Badger and Weasel amount to 130 and 470 $\mu\text{g/kg}$, respectively. Thus the MPC₅s for the selected mammals are within the range of the ones for the eight selected birds.

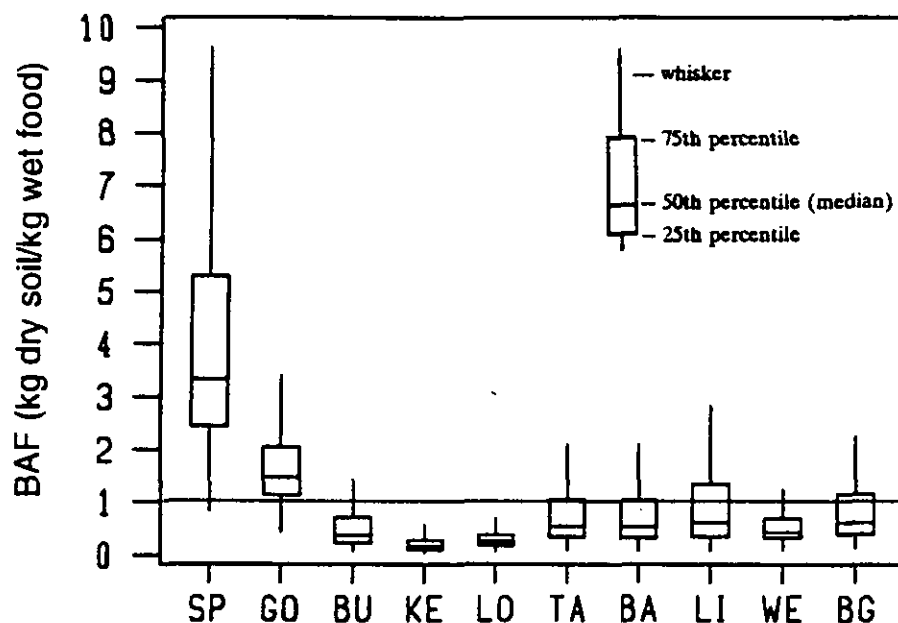


Figure 6. Box and Whisker plot of distributions of the total bioaccumulation factor for DDT from soil to food of the selected top predator species. The 25, 50 and 75th percentiles are shown, together with the range within 1.5 x standard deviation (whiskers).

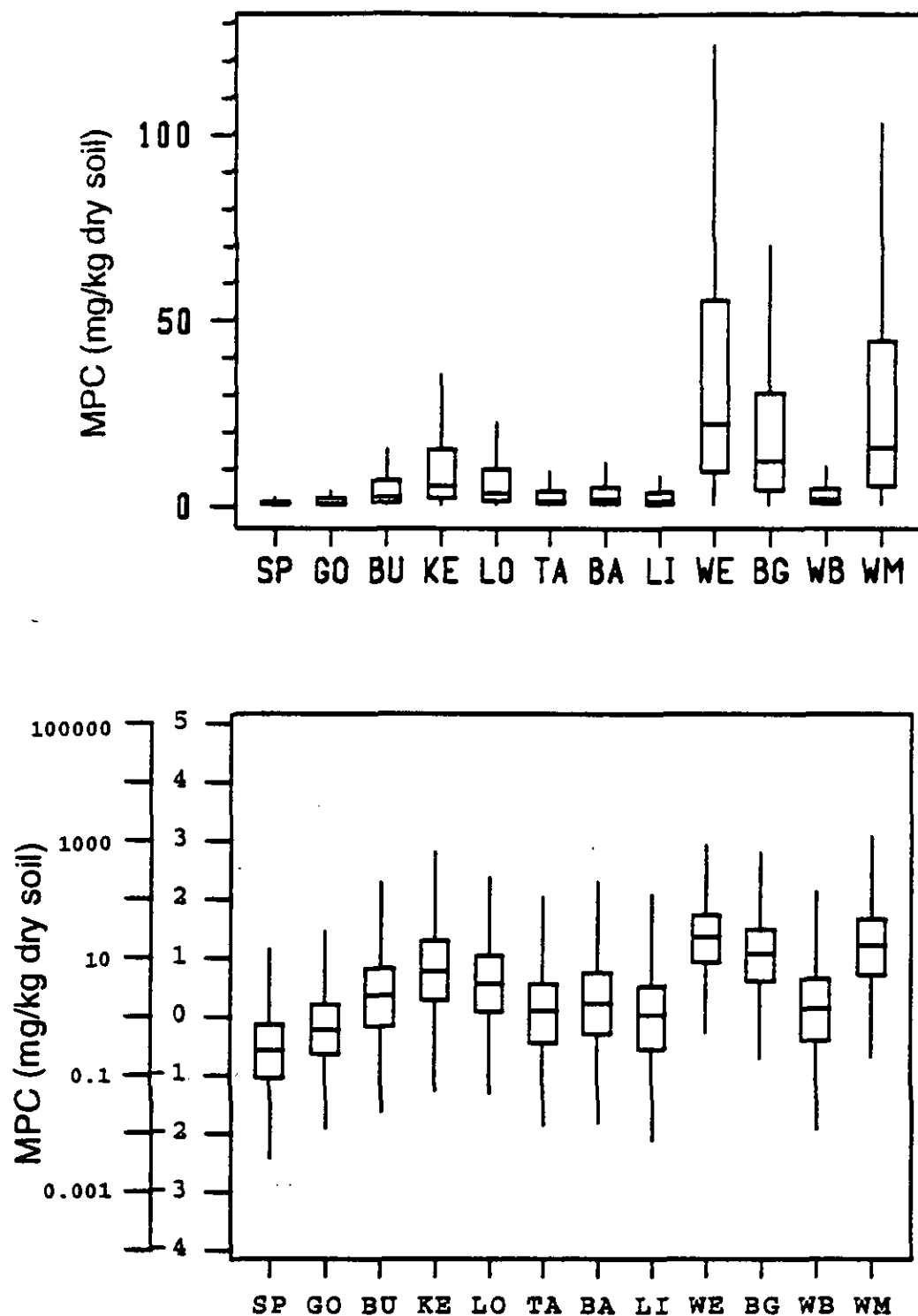
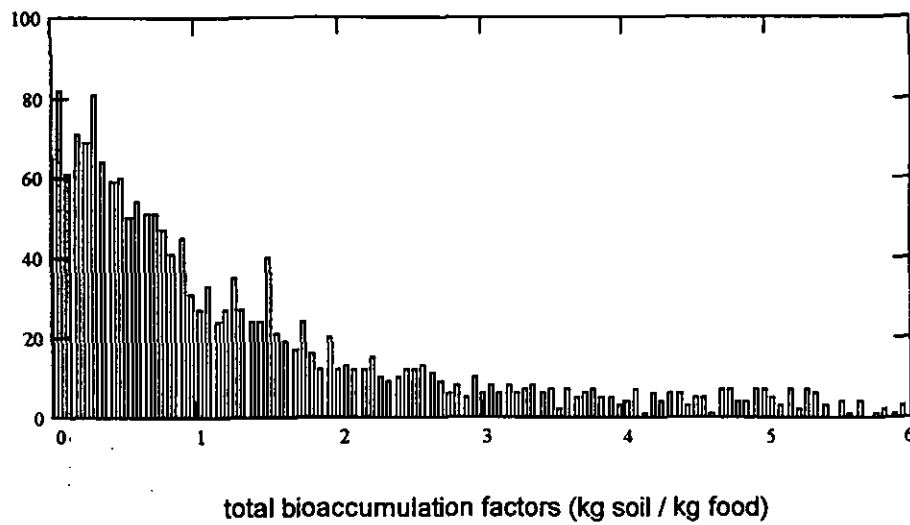
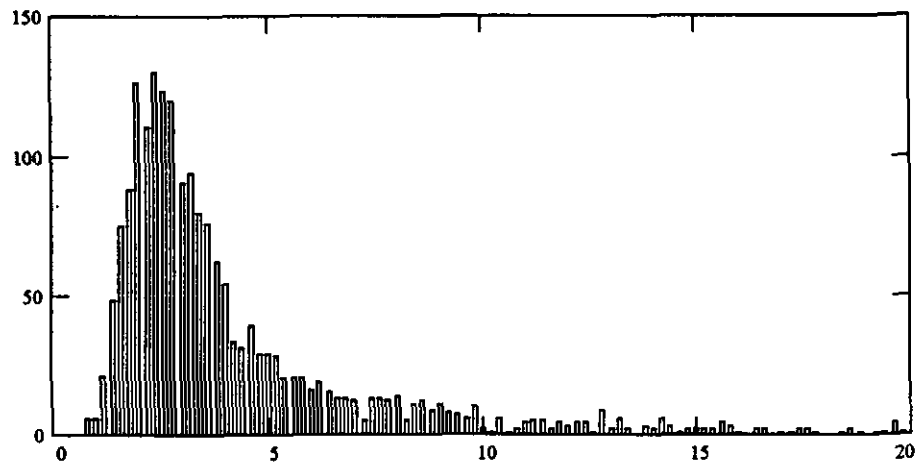


Figure 7. Box and Whisker plot of distributions of the MPCs for DDT in soil based on secondary poisoning of the selected top predator species, worm-eating bird (WB) and worm-eating mammal (WM). Above: linear scale, below: log scale. The 25, 50 and 75th percentiles are shown, together with the range.

NOECs (mg DDT / kg food)



total bioaccumulation factors (kg soil / kg food)



MPCs (mg DDT / kg soil)

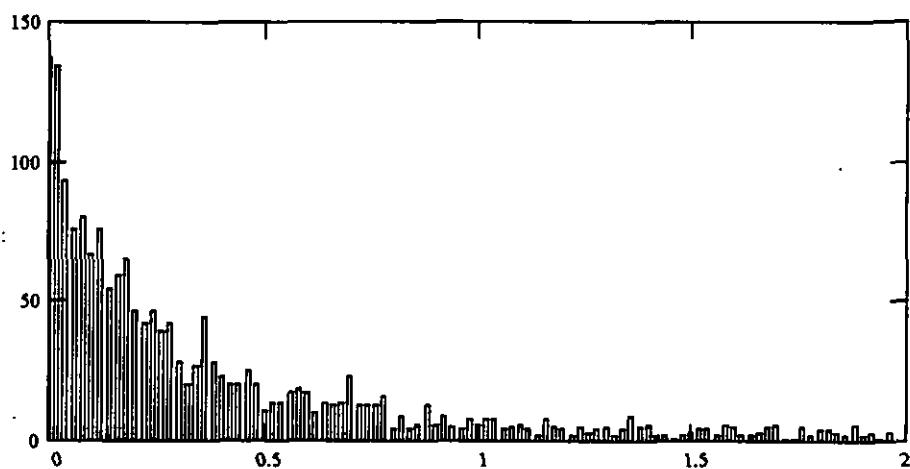


Figure 8: Frequency distributions of NOECs, total bioaccumulation factors from soil to food and MPCs of DDT for the Sparrowhawk.

6.2.3 Lindane

Food chains

The food chains *soil* → *seed* → *bird* and *soil* → *seed* → *mammal* lead to the highest mean exposure to lindane, with BAF₅₀s of 0.27 and 0.29, respectively. The lowest median BAF₅₀s are estimated for the food chains *soil* → *leaf* → *bird* and *soil* → *leaf* → *mammal*, with values of 0.016 and 0.017, respectively.

The scarcity of NOECs for lindane for birds and mammals makes it impossible to carry out a reliable calculation of MPCs. Besides NOECs, more BCFs and BAFs are required. The MPCs derived for top predators should therefore be treated with caution. The MPC₅s for birds and beast of prey range from 0.005 and 3.6 mg/kg and 0.055 to 51 mg/kg, respectively. The food chains including insects are critical for secondary poisoning of birds and mammals by lindane.

Selected species

The total bioaccumulation of lindane from soil to food is low in the raptorial birds consuming only vertebrates. BAF₅₀s in these species vary between 0.027 (Kestrel) and 0.13 (Sparrowhawk). A higher BAF₅₀ is found for the Little Owl (0.19). This is due to the fact that the Little Owl consumes earthworms and caterpillars.

A considerable difference is found between the extent of the accumulation of lindane in the food of the two mammalian species. The BAF₅₀ for the food of the Badger is 0.83, which is a factor 18 higher than the one for the food of the Weasel.

The low bioaccumulative potential of lindane in the food of the top predators preying mainly on birds and mammals is also demonstrated by the BAF₇₅s. These values range from 0.06 to 0.19 for the avian species, and for the Weasel a value of 0.08 is found. Much higher BAF₇₅s are found for Little Owl (0.48) and Badger (1.24).

The lowest MPC₅ for the raptorial birds is derived for the Little Owl (43 µg/kg), followed by the Sparrowhawk (340 µg/kg). The highest MPC₅s are found for Kestrel and Long-eared Owl, species feeding almost exclusively on herbivorous mammals.

The MPC₅ for the Badger amounts to 360 µg/kg, which is a factor 26 lower than the MPC₅ for the Weasel.

6.2.4 PCP

Food chains

The food chain *soil* → *worm* → *mammal* is the most critical one for PCP exposure of top predators feeding on birds and mammals, but even then this exposure is quite low with a BAF_{50} of 0.125.

Reliable MPC_5 s for PCP can not be calculated with the proposed method due to the shortage of data concerning NOECs, BCFs and BAFs. In analogy with lindane, MPC_5 s for PCP have to be based on NOECs derived with the modified EPA method.

The differences among the MPC_5 s based on exposure through the food chains are large, ranging from 1.7 to 14286 mg/kg for birds of prey and from 0.63 to 7857 mg/kg for beast of prey. The lowest MPC_5 s are derived for the food chains *soil* → *worm* → *mammal* for both birds and beast of prey.

Selected species

In the avian species BAF_{50} s range from 0.008 for the Sparrowhawk to 0.072 for the Kestrel. However, the calculated BAF_{50} for the Little Owl is much higher (0.20) due to direct consumption of earthworms.

A considerable difference between BAF_{50} s is found for both mammalian species, with values of 0.037 for the Weasel and 0.60 for the Badger.

The BAF_{75} values of top predators consuming mainly birds and mammals are all far below one, suggesting that the exposure to PCP can be considered negligible in these species.

The MPC_5 s of the species consuming a quantitatively important portion of invertebrates like Little Owl and Badger are much lower than the ones of the other selected birds and beast of prey species. The MPC_5 for the Little Owl amounts to 0.09 mg/kg, whereas the MPC_5 s among the other avian species varies between 1.5 (Barn Owl) and 23 mg/kg (Goshawk). The MPC_5 for the Badger is estimated at 0.032 mg/kg, being a factor 56 smaller than the MPC_5 for the Weasel.

6.2.5 Methyl mercury

Food chains

It should be realized that a reliable calculation of the accumulation of MeHg from soil to food of the top predators is hampered by the fact that only one study was

suitable to supply input data for bioconcentration of MeHg from soil into invertebrates. Bioaccumulation along the food chains *seed* → *mammal or bird* can not be calculated either. The BAF_{50} s range from 0.056 for the food chain *soil* → *leaf* → *bird* to 28 for the food chain *soil* → *worm* → *mammal*.

As stated above risk of secondary poisoning of top predators for MeHg can only be estimated for four of the eight food chains. The lowest MPC_5 s are based on the food chain *soil* → *worm* → *mammal* with values of 1.5 and 0.73 $\mu\text{g}/\text{kg}$ for raptorial birds and mammals, respectively.

Selected species

The calculated BAF_{50} values exceed one for both beast of prey species and for five of the bird of prey species. The exposure to MeHg is the highest in species consuming either many invertebrates (Little Owl, Badger) or relatively many insectivorous mammals (Barn Owl, Buzzard). The variation in the MeHg- BAF_{50} among the avian species is high, ranging from 0.44 for the Long-eared Owl to 8.23 for the Little Owl. BAF_{50} s for Weasel and Badger amount to 1.5 and 4.0, respectively.

The MPC_5 for the selected birds of prey ranges from 3.3 $\mu\text{g}/\text{kg}$ for the Little Owl to 70 $\mu\text{g}/\text{kg}$ for the Long-eared Owl. The Barn Owl has a MPC_5 of 4.0 $\mu\text{g}/\text{kg}$ and is the most critical species among the selected birds preying almost entirely on birds and mammals. The MPC_5 for the Badger (4.0 $\mu\text{g}/\text{kg}$) is a factor three lower than the MPC_5 for the Weasel.

6.2.6 Cadmium

Food chains

The food chains *soil* → *worm* → *bird/mammal* are by far the most critical pathways for exposure of birds and beast of prey to Cd, whereas bioaccumulation in the food chains *soil* → *leaf* → *bird/mammal* is negligible.

MPC_5 s for Cd based on exposure through one of the eight food chains range from 0.080 to 3.6 mg/kg for birds of prey and from 1.5 to 48 mg/kg for beast of prey. The lowest MPC_5 s refer to the food chains worm-bird. MPC_5 s for top predators feeding exclusively on worm-eating mammals are slightly higher.

Selected species

The total BAF_{50} for Cd in the food of the raptorial bird species ranges from 0.005 and 1.04. The highest BAF_{50} is found for the Little Owl due to the consumption of

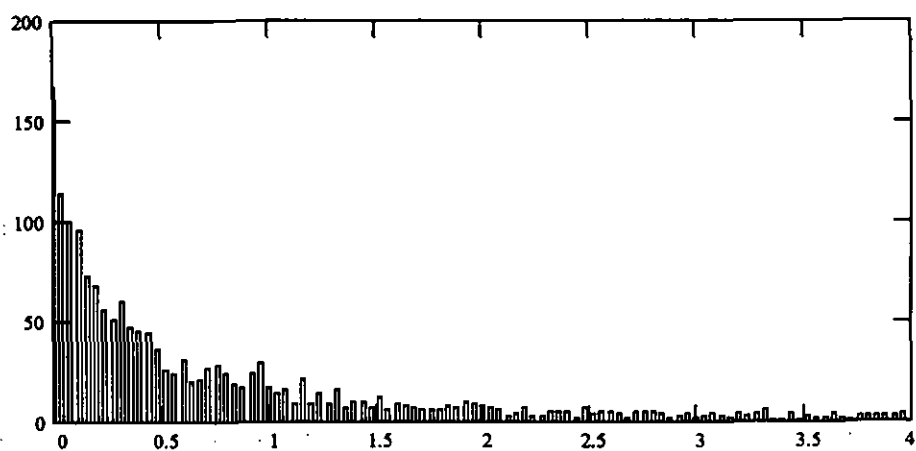
relatively many earthworms by this bird. The BAF_{50} for the raptorial birds feeding mainly on birds and mammals varies from 0.005 for the Long-eared Owl and the Kestrel to 0.056 for the Sparrowhawk. The BAF_{50} for the Badger is 1.44, which is a factor 120 higher than the BAF_{50} calculated for the Weasel.

The variation among the selected species with respect to the MPC appears to be comparable with the variation in total bioaccumulation. The Little Owl is the most critical species for Cd, whereas the Sparrowhawk is the most critical species when only vertebrate-eating species are considered. The MPC_5 s for the Little Owl and the Sparrowhawk amount to 0.012 and 0.30 mg/kg, respectively. The highest MPC_5 s are found for the Long-eared Owl and the Kestrel with values of 2.4 and 2.0 mg/kg, respectively. Both species prey mainly on herbivorous mammals.

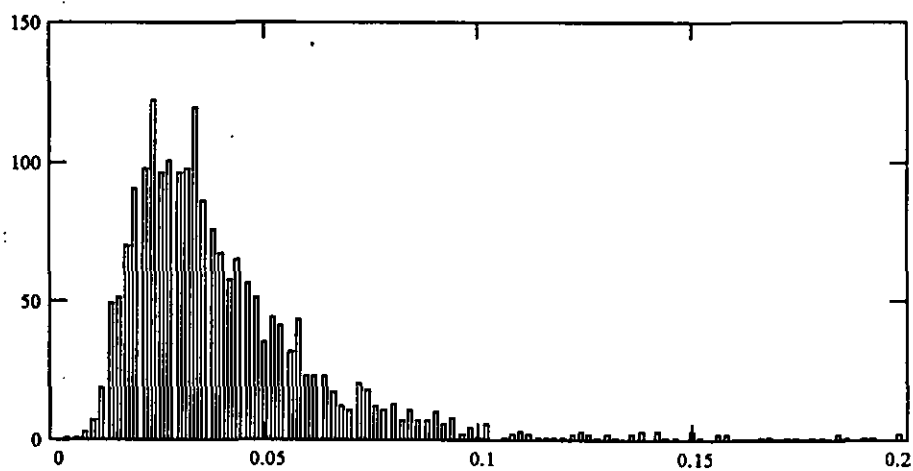
Figure 9 shows the distributions of corrected NOECs, BAFs in its food, and MPCs in the soil for the Barn Owl. These distributions have the shape of log-logistic distributions. However, this can not be seen easily in case of the corrected NOECs and the MPCs due to the extreme skewness of the distributions.

The Badger has a MPC_5 of 0.16 mg/kg, which is a factor 150 lower than the MPC_5 calculated for the Weasel.

NOECs (mg Cd / kg food)



total bioaccumulation factors (kg soil / kg food)



MPCs (mg Cd / kg soil)

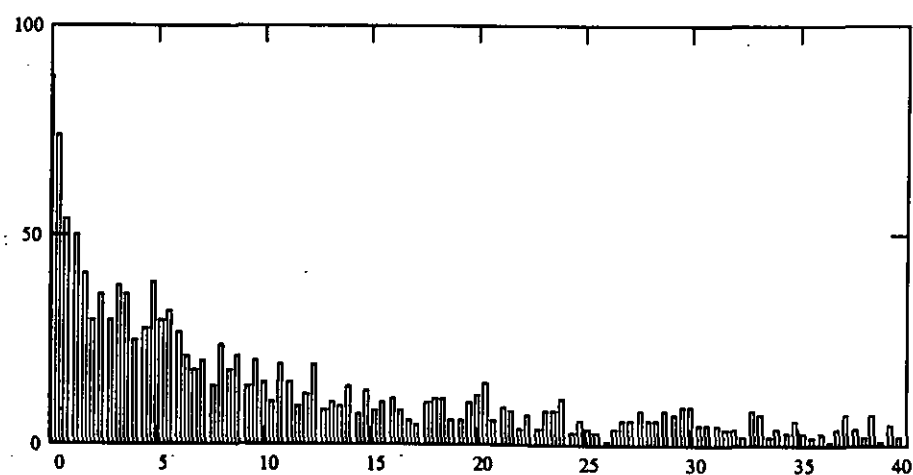


Figure 9. Frequency distributions of NOECs, total bioaccumulation factors from soil to food and MPCs of cadmium for the Barn Owl.

Table 19. BAF₅₀ values ($\text{kg}_{\text{dry soil}}/\text{kg}_{\text{wet tissue}}$) of chemicals for eight food chains (A) and from soil to the food of the selected top predator species (B). The highest BAF₅₀ value of each chemical is shown in bold for both bird of prey and beast of prey.

A: Food chains

Food chain	BAF ₅₀					
	DDT	dieldrin	lindane	PCP	MeHg	Cd
soil → leaf → bird	0.21	0.048	0.016	0.011	0.056	0.0037
soil → seed → bird	4.37	-	0.27	0.00070 ^b	-	0.025
soil → worm → bird	0.73	0.43	0.045 ^{bc}	0.022	7.8	0.20
soil → insect → bird	2.6	0.70	0.067	0.00079	-	0.029
soil → leaf → mammal	0.067	0.025	0.017	0.065	0.20	0.0024
soil → seed → mammal	1.47	-	0.29	0.0040 ^b	-	0.016
soil → worm → mammal	0.24	0.23	0.049 ^{bc}	0.125	28	0.13
soil → insect → mammal	0.86	0.37	0.072	0.0045	-	0.018

^b not stochastic because one value is available

^c QSAR for bioconcentration in earthworms

B: Selected species

Species	BAF ₅₀					
	DDT	dieldrin	lindane	PCP	MeHg	Cd
<u>Birds of prey</u>						
Sparrowhawk	3.34	0.31	0.13	0.008	3.15	0.056
Goshawk	1.47	0.11	0.10	0.009	0.60	0.019
Buzzard	0.36	0.071	0.048	0.060	5.22	0.022
Kestrel	0.16	0.031	0.027	0.072	0.86	0.006
Long-eared Owl	0.25	0.034	0.037	0.046	0.44	0.005
Tawny Owl	0.55	0.093	0.041	0.025	3.63	0.023
Barn Owl	0.52	0.11	0.053	0.070	7.94	0.036
Little Owl	0.62	0.27	0.19	0.20	8.23	1.04
<u>Beasts of prey</u>						
Weasel	0.42	0.057	0.047	0.037	1.53	0.012
Badger	0.63	0.26	0.83	0.60	4.03	1.44

Table 20A. MPC₅ values (mg_{chem}/kg_{dry soil}) of chemicals for birds and beasts of prey^a based on exposure through eight different food chains. The lowest MPC₅ value of each chemical is shown in bold for both bird of prey and beast of prey.

Food chain	MPC ₅					
	DDT	dieldrin	lindane	PCP	MeHg	Cd
<u>Birds of prey</u>						
soil → leaf → bird	0.21	0.21	0.12	22	0.54	2.3
soil → seed → bird	0.018	-	0.084	14286 ^b	-	0.44
soil → worm → bird	0.10	0.059	3.6 ^{bc}	10	0.0046	0.080
soil → insect → bird	0.011	0.036	0.0056	150	-	0.40
soil → leaf → mammal	0.60	0.75	0.11	3.9	0.16	3.6
soil → seed → mammal	0.047	-	0.78	2500 ^b	-	0.68
soil → worm → mammal	0.26	0.20	3.3 ^{bc}	1.7	0.0015	0.124
soil → insect → mammal	0.030	0.098	0.0050	26	-	0.61
<u>Beasts of prey</u>						
soil → leaf → bird	3.8	0.23	1.5	10	0.32	37
soil → seed → bird	0.37	-	1.1	7857 ^b	-	7.2
soil → worm → bird	2.0	0.062	56 ^{bc}	4.3	0.0028	1.5
soil → insect → bird	0.19	0.038	0.070	66	-	6.4
soil → leaf → mammal	9.0	0.67	1.2	1.5	0.076	48
soil → seed → mammal	0.76	-	0.83	1375 ^b	-	9.4
soil → worm → mammal	4.2	0.18	51 ^{bc}	0.63	0.00073	1.9
soil → insect → mammal	0.43	0.085	0.055	10	-	8.3

^a The total correction factor used for extrapolation of the NOECs from laboratory studies is 0.24 for bird-eating birds, 0.21 for mammal-eating birds, 0.17 for bird-eating mammals and 0.15 for mammal-eating mammals.

^b not stochastic because one value is available

^c QSAR for bioconcentration in earthworms

Table 20B. MPC₅ values (mg_{chem}/kg_{dry soil}) of chemicals based on the risk of secondary poisoning of selected top predator species. The lowest MPC₅ values of each chemical are shown in bold for both birds of prey and beasts of prey.

Species	MPC ₅					
	DDT	dieldrin	lindane	PCP	MeHg	Cd
<u>Birds of prey</u>						
Sparrowhawk	0.015	0.078	0.34	11	0.011	0.30
Goshawk	0.036	0.21	0.36	23	0.055	0.75
Buzzard	0.090	0.47	0.66	1.6	0.007	0.71
Kestrel	0.25	0.53	0.85	2.8	0.038	2.0
Long-eared Owl	0.20	0.60	0.96	5.0	0.070	2.4
Tawny Owl	0.063	0.31	0.70	2.9	0.008	0.55
Barn Owl	0.070	0.29	0.47	1.5	0.004	0.45
Little Owl	0.040	0.13	0.043	0.090	0.0033	0.012
<u>Beasts of prey</u>						
Weasel	1.6	0.47	9.4	1.8	0.013	24
Badger	0.75	0.13	0.36	0.032	0.0040	0.16

7. DISCUSSION

The proposed model is suitable for the calculation of acceptable concentrations of contaminants in the food and in the soil (MPCs) for top predators, provided sufficient data are available concerning NOECs, BCFs and BAFs. This is the case for Cd, methyl mercury, DDT and to a lesser extent for dieldrin. For these chemicals stochastic NOECs are used instead of a constant NOEC derived with the refined method of Aldenberg and Slob. For lindane and PCP less than four NOECs are available for birds as well as for mammals which means that the EPA procedure for the derivation of a NOEC for birds and a NOEC for mammals in general has to be applied. Thus the uncertainty in the estimation of MPC_{5s} for lindane and PCP is indicated by incorporation of the EPA safety factor. The calculated variation in the MPC distributions of both chemicals is not realistic due to the absence of information about the variation in both NOECs and many BCFs and BAFs.

It should be noticed that there are also top predators consuming plant parts or invertebrates next to birds and mammals. Little Owl and Badger are examples for these types of top predator, which do not belong to the four types considered in this report. These two species have a position between typical top predators (bird- and/or mammal-eaters) and worm-eaters. This implies that for the derivation of MPCs for secondary poisoning by chemicals attention may be confined to strict bird and mammal-eaters and strict worm-eaters.

7.1 MPCs for specific locations, life-stage and season

Mean values are used for the correction factors in order to calculate soil quality criteria for general protection. Moreover, for the selected species food choice is averaged over a year and over locations. However, food choice may vary within a species due to location, season and life-stage. It is possible to adjust the model to specific conditions by varying the input data.

Calculation of secondary poisoning of Barn Owls is based on an average yearly diet of several locations in The Netherlands. However, the risk of secondary poisoning may differ considerably among locations due to variation in food choice. The food choice depends on the abundance of prey species. De Bruijn (1979) demonstrated that the frequency of small insectivorous mammals in the diet of Barn Owls in the Netherlands may fluctuate between 7 and 43% on a weight basis. The Common Vole (*Microtus arvalis*) is the preferred prey species of Barn Owls but in years with a scarcity of voles more shrews are consumed which may be expected to lead to a higher exposure of the selected contaminants.

At the Brabantse Kempen, which encloses the Budel area, the portion of shrews is the highest of all locations studied by De Bruijn (1979) and amounts to 42.2%. In the average diet of the Barn Owl shrews make up 24.1%. As a consequence of this diverging diet the calculated MPC₅ (0.30 mg/kg) for Cd would be lower at Budel than the average MPC₅ for the Barn Owl (0.45 mg Cd/kg).

To guarantee that a target species is sufficiently protected against secondary poisoning, detailed information is needed on metabolic rate and species sensitivity as well. However, the available data on species sensitivity provide no decisive answer to the necessity to correct for species sensitivity. On the other hand differences in energy expenditure between laboratory animals and free-living animals are clearly demonstrated and can be included in the model. Birds and mammals need more energy during migration, cold and reproductive periods. It may be more realistic to use sustained metabolic rate instead of average metabolic rate for these more extreme conditions. In that case 0.25 can be applied instead of 0.4 as a correction factor for metabolic rate. The result is a reduction of 37.5% in both corrected NOECs and MPC₅s.

The Cd accumulation observed in free-living shrews and voles (Hunter et al., 1987c, Ma et al., 1991) is much higher than the one in experiments with laboratory mammals (Appendix H9). It is possible that the field metabolic rate of free-living shrews and voles is substantially higher than the average metabolic rate for laboratory mammals with similar body weight. Direct uptake of Cd from the soil by shrews and voles may be an important pathway for Cd accumulation as well.

For an evaluation of the effects of environmental pollution on birds and mammals the specific life cycle and food requirements should be taken into account. Winter and Streit (1992) demonstrated that omnivorous passerine birds may be at risk for secondary poisoning by persistent organochlorines. The critical period for Great Tits (*Parus major*) is the early life stage due to transfer from the mother bird and due to contaminated food (caterpillars) in combination with low fat reserves.

Geyer et al. (1993) demonstrated that a large fat reserve protects animals against the toxic action of lipophilic and persistent contaminants. Single oral 30-day LD50 values of 2,3,7,8-TCDD in different species and strains of mammals correlated positively with their total fat content (ranging from 4.5 to 20.2%). Differences in sensitivity between and within species can possibly be explained by differences in total fat content. This stresses the need to include determinations of total body fat content of the organisms in toxicity tests with lipophilic chemicals. Bogan and Newton (1977) demonstrated the hazard of DDE poisoning for birds in poor condition. Sparrowhawks with less than 2% body fat contained much higher DDE concentrations in the brain than individuals with body fat between 2 and 9%.

7.2 Cd levels in free-living Barn Owls

Unfortunately, there are only few field data available which can be used for validation of the model calculations. Denneman and Douben (1993) measured cadmium concentrations in feathers and target organs of Barn Owls in The Netherlands in order to investigate whether the current Cd level may play a role in the population decline of this species. Barn Owls in the Cd contaminated area at Budel have significantly higher Cd concentrations in kidney and liver than Barn Owls from uncontaminated areas, but the accumulation of Cd is low. The highest Cd concentrations in kidney and liver of Barn Owls from the Budel area amounted to 12.8 and 3.1 mg/kg dry weight, respectively, being far below the Cd level of 200 mg/kg dry weight causing pathological kidney damage in pigeons (Hutton, 1980). Denneman and Douben (1993) concluded that Cd apparently poses no large scale threat to Dutch Barn Owls. On the other hand, Cd levels in kidneys of its major prey species, the Common Shrew (*Sorex araneus*) frequently exceed the critical level (Ma et al., 1991). The following estimations can be made with our model applying the Cd concentration reported for the Budel soil (2.9 mg/kg). The probability of exceeding the Cd-NOECs of Common Shrews and Barn Owls amounts to 94% and 18%, respectively. The MPC₅ for Cd can be estimated at 0.05 and 0.45 mg Cd/kg soil for Common Shrew and Barn Owl, respectively (see Table 20). As pointed out earlier in this report it may be preferred to apply the diet of Barn Owls at the Brabantse Kempen, resulting in the estimation of a higher risk namely 24.7%. This diet combined with sustained metabolic rate conditions instead of average field metabolic rate may be considered as a worst-case for Barn Owls at Budel leading to a risk of 32.2% and a concomitant MPC₅ of 0.20 mg Cd/kg soil.

It has to be considered that validation of model calculations is hampered by the fact that the Cd concentrations in the soil are often not constant within territories of birds and beasts of prey. In the Budel area Cd concentrations in the soil vary between 0.4 and 3 mg/kg over a short range (Gorree and Tamis, 1993). Validation is probably more difficult for bird-eating raptors than for mammal-eating raptors. Bird-eating raptors are affected by soil contaminants over much wider areas than mammal-eating raptors which tend to be affected mainly in areas where pesticides are applied directly (Newton, 1979).

Newton (1979) reported that among raptorial birds in any given area, mammal-eaters always contain lower organo-chlorine levels than bird-eaters. This was reflected by DDE levels in eggs of various predatory birds on the Canadian Prairies in the nineteen sixties with about 20 mg DDE/kg in the bird-eating Peregrine Falcon and Merlin (*Falco columbarius*) as compared to less than 2 mg DDE/kg in Kestrel and Long-eared Owl. A study on three *Accipiter* species in North America demonstrated

that the Sharp-shinned Hawk (*Accipiter striatus*) feeding entirely on insectivorous birds had the highest DDE concentrations in its eggs. The Goshawk feeding mainly on herbivorous mammals and birds had the lowest DDE levels, while the Cooper's Hawk (*Accipiter cooperii*) was intermediate in both DDE levels and food choice. Newton (1979) concluded in his review that raptors are particularly vulnerable to DDE because of their positions high in food chains and because of their high sensitivity. Accipitridae and Falconidae appear to be more sensitive to DDE in their eggs than some other bird-families. A DDE concentration of 4-5 mg/kg in fresh eggs coincided with at least 15% egg-shell thinning in raptors, whereas about 10% and 1% thinning was found in songbirds and gamebirds, respectively. Field data gave no indications for variation among raptorial bird species in six genera in sensitivity to DDE concentrations in their eggs. In general, population declines occurred at 16-20 percent shell thinning.

7.3 Bioaccumulation of Cd

It seems a simplification to use BAFs for cadmium. There are indications that internal Cd levels of free-living mammals and birds continue to increase during lifetime. This was found for Moles (*Talpa europaea*) (Pankakovski et al., 1993), Common Shrews (Hunter et al., 1989), Badger (Ma and Broekhuizen, 1989) and Great Skua (*Catharacta skua*) (Furness and Hutton, 1979). However, in laboratory experiments contradictory results are found. Steady state levels of Cd were found in the rat by Loeser and Lorke (1977), Bernard (1981) and Kajikawa (1981) in feeding studies lasting 12, 36, and 45 weeks, respectively. On the other hand, Cd plateau levels were not reached in feeding experiments with Laboratory Rat (*Rattus norvegicus*) (Suguwara and Suguwara, 1974), Laboratory Mouse (*Mus musculus*) (Watanaba, 1986) and Chicken (*Gallus domesticus*) (Leach et al., 1979) in experiments of 41, 64, 48 weeks, respectively. The accumulation of Cd with increasing age is difficult to include in a model, especially because the age of prey animals has to be known and it may be assumed that a large portion of these animals has not reached adulthood. French et al. in Golley et al. (1975) report a life expectation for the mammalian families of Muridae (mice), Microtinae (voles) and the mammalian order of Insectivora (shrews and moles) of 1.8, 3.1 and 7.4 months, respectively. The average age of Common Shrew populations depends on the season and varies from 3 to 10 months (Crowcroft, 1957).

7.4 QSARs as an alternative for experimental BAFs

QSARs for accumulation of Cd and organic compounds in earthworms offer a workable alternative in the absence of reliable experimental data for bioaccumulation.

In the literature QSARs are also available for organic chemicals in plants, and birds and mammals. However, these QSARs are not reliable. This can at least partly be ascribed to the relatively high biodegradation capacity in the case of mammals. Nevertheless these QSARs can be used as a tool for screening the potential of lipophilic compounds in plants, birds and mammals.

7.5 Importance of including secondary poisoning in setting quality objectives

Soil quality objectives are supposed to provide enough protection to both soil organisms and birds and mammals. For some chemicals MPC₅s derived for secondary poisoning may be lower than the MPC₅s derived for direct exposure of soil organisms. Romijn et al. (1991b, 1994) gave attention to the food chain *soil* → *worm* → *bird/mammal* because it may be expected that for many chemicals this may be the most critical pathway for secondary poisoning of birds and mammals in a terrestrial ecosystem. They demonstrated that secondary poisoning of worm-eating birds and mammals may be more critical than direct exposure of soil organisms. This was the case for Cd and methyl mercury, but not for four organic compounds: DDT, dieldrin, lindane and PCP. The same conclusions can be drawn from the work of Van de Plassche (1994). The MPC₅s for worm-eaters derived by Van de Plassche (1994) are lower than the ones from Romijn et al. (1991b, 1994) due to the correction for caloric content and using a mean BCF of one instead of experimental values for organic compounds. In the present report it is tried to further improve the risk assessment on secondary poisoning within terrestrial food webs by application of two more correction factors, namely assimilation efficiency and metabolic rate, in combination with a stochastic treatment of NOECs and BCFs. This resulted in MPC₅s for worm-eating birds and mammals that are generally lower than the ones from Van de Plassche (1994) (see table 21). It follows that secondary poisoning of worm-eaters may also be more critical than direct exposure of soil organisms for organic compounds like PCP and dieldrin.

It is necessary to pay attention to exposure of top predators to soil contaminants when MPC₅s derived for worm-eating birds and mammals are not low enough for an acceptable protection of certain top predators. Therefore MPC₅s for worm-eaters are compared with the (lowest) MPC₅s for top predators in Table 21. The MPC₅s of PCP and Cd are lower for worm-eaters than for the most critical food chains for top predators, and the MPC₅s for methyl mercury are comparable. Worms form part of the most critical food chains for these three chemicals. Apparently, the step *small bird/mammal* → *bird or beast of prey* does not seriously increase the risk of secondary poisoning by these chemicals. The risk for secondary poisoning by DDT and lindane is higher for insectivorous birds and mammals than for worm-eating birds and

mammals (data not shown). This can be ascribed to higher BCFs (with higher variation) of these compounds for insects than for earthworms. The MPC₅s of DDT, dieldrin and lindane are lower for the critical food chain *soil → insect → bird → top predator* than for the food chain *soil → worm → bird/mammal*.

There are few terrestrial top predator species that are exposed through a single food chain consisting of either earthworms or insects. In the food webs of the eight selected strictly vertebrate-eating species the percentage earthworms and insects of the total food types is quite small, ranging between 0.6 and 16.5% and between 0.1 and 18.6%, respectively. This implies that the actual risk of secondary poisoning by soil contaminants for top predator species is generally much lower than the risk based on the most critical food chain. The MPC₅s of dieldrin and lindane based on the exposure of the selected species are all above the MPC₅s of the worm-eaters. DDT is the only compound for which some top predator species have a lower MPC₅ as compared to the MPC₅s for worm-eaters. Thus, in order to get insight into the probability of the risk of secondary poisoning of top predators, a risk assessment for certain species can be recommended, after the most critical food chain is identified.

Secondary poisoning of a bird-eating birds exposed to DDT through the food chain *soil → insect → bird* and a species like the Sparrowhawk is about equally critical than direct exposure of soil organisms to DDT. However, it should be noted that calculations are made for average conditions with respect to both food choice and correction factors. In worst-case situations DDT-MPC₅s for birds feeding on small birds may be expected to be even lower than the ones for soil organisms.

It may be concluded that in the derivation of soil quality objectives secondary poisoning of birds and mammals has to be taken into consideration next to direct exposure of soil organisms. As a standard procedure attention should be given to worm-eating birds and mammals. For highly lipophilic compounds a risk assessment should be made for top predators predating on birds and mammals eating worms and/or insects.

7.6 Alternative procedures

It should be realized that a proper comparison between MPC₅s for direct effects on soil organisms and indirect effects on birds and mammals is hampered by the scarcity of data. This especially refers to toxicity data for DDT, methyl mercury and lindane concerning soil organisms (Van de Plassche, 1994), and both toxicity and bioaccumulation data for lindane and PCP concerning birds and mammals.

It is obvious that the calculated MPCs for secondary poisoning strongly depend on the methodological choices and assumptions that are made. The most important choices

and their alternatives are:

1. For BCFs and BAFs experimentally derived values are used, alternatively QSARs can be used. Van de Plassche (1994) used for all organic compounds for earthworms a mean and maximum BCF of 1 and 10, respectively.
2. For the toxicity of a chemical (NOECs) concentrations in food are used, alternatively critical concentrations in target organs and tissues can be used.
3. For diet and correction factors (metabolic rate), average conditions are applied, alternatively (realistic) worst-case conditions can be used.
4. For the corrections constant values are used, alternatively stochastics can be used
5. No safety factors are applied for potentially important aspects (species sensitivity, pollutant assimilation efficiency), for which insufficient data are available, however, this may be done.
6. The level of protection for critical or attention species is arbitrary. In the present report the 5th percentile of the MPC distribution (MPC_5) is selected for this purpose.

Table 21. Comparison of estimated MPC_{5s} (mg_{chem}/kg_{dry soil}) values based on the risk of secondary poisoning of top predators and worm-eaters (birds and mammals). For top predators, the lowest MPC_{5s} among separate food chains^a indicated by CF: critical food chain, and among the selected species indicated by CS: critical species are listed. MPC_{5s} of soil organisms (mg/kg soil) are from Van de Plassche (1994) and Slooff et al. (1993). The MPC_{5s} calculated with the present model are compared with the MPC_{5s} reported by Van de Plassche (1994).

The lowest MPC_{5s} among birds and among mammals are printed in bold. The overall lowest MPC_{5s} are underlined.

			MPC ₅					
			DDT	dieldrin	lindane	PCP	MeHg	Cd
MPC_{5s} from Table 20A,B of present report								
birds	worm-eater		0.16	0.050	0.025	0.037	0.0016	<u>0.0019</u>
	top predator	CF	0.011	<u>0.036</u>	<u>0.0050</u>	1.7	0.0015	0.080
	top predator	CS	0.015	0.078	0.043	0.090	0.0033	0.012
mammals	worm-eater		2.7	0.055	0.33	<u>0.017</u>	0.00080	0.032
	top predator	CF	0.19	0.038	0.055	0.63	<u>0.00073</u>	1.5
	top predator	CS	0.78	0.13	0.36	0.032	0.0040	0.16
MPC_{5s} in Van de Plassche et al. (1994)								
birds	worm-eater		0.048	0.067	0.18	5.8	0.0026 ^b	0.0035
mammals	worm-eater		1.7	0.084	0.58	1.3	0.0027 ^b	0.20
soil organisms			<u>0.01</u>	0.05	<u>0.005</u>	0.17	0.4	0.27

^a Eight food chains were considered: connecting the soil compartment with one of the four components from the first trophic level (plant leaves, plant seeds, earthworms, insects) followed by one of the two components from the second trophic level (birds, mammals).

^b A combined data-set of birds and mammals resulted in a MPC₅ value of 0.0033 and is proposed as final value in Van de Plassche (1994).

8. CONCLUSIONS

A model is developed for standard setting based on the risk of secondary poisoning for birds and mammals, including top predators. The model is aimed at deriving general protection levels, but can be adjusted to specific locations, seasons or life-stages by varying the food choice. It is possible to make calculations for worst-case conditions, for example by application of a correction for sustained metabolic rate instead of average metabolic rate.

In order to extrapolate from laboratory conditions to field conditions corrections have to be applied for caloric content and assimilation efficiency of food types, and for metabolic rates. Corrections for caloric content and metabolic rates are quantitatively more important than correction for food assimilation efficiency (see Table 22). These three correction factors should be generally applied in procedures for risk-assessment of secondary poisoning by potentially bioaccumulative compounds, amongst others for worm eating birds and mammals.

Table 22. A survey of (range of) values of the quantifiable correction factors (used in equations 2-2 and 6-1) which are applied to types^a and selected species of top predators.

Top predators	EMR/FMR	CC _{field} /CC _{lab}	FAE _{field} /FAE _{lab}
<u>Type^a</u>			
Birds	0.4 (0.25 ^b)	0.52 - 0.58	1.02
Mammals	0.4 (0.25 ^b)	0.42 - 0.47	0.92
<u>Selected species</u>			
Birds	0.4 (0.25 ^b)	0.43 - 0.58	0.93 - 1.02
Mammals	0.4 (0.25 ^b)	0.42 - 0.44	0.87 - 0.92

^a Only bird-eating and mammal-eating types are considered

^b this value can be applied as a worst case (more extreme energy demanding periods)

It appears that biomagnification (BAFs > 1) is not a general phenomenon in terrestrial organisms. However, occasional high BAFs are found e.g. for DDT in birds, MeHg in mammals and earthworms, and Cd in worms, isopods and spiders. The variation in BAF for a certain chemical is considerable among invertebrates and

also between birds and mammals. The variation in diets among raptorial species therefore leads to pronounced differences in exposure to contaminants in the food. The scarcity of data on bioaccumulation, especially in the case of PCP and lindane, is a great drawback in the estimation of the risk of secondary poisoning for both birds and mammals.

The food chain *soil* → *worm* → *bird/mammal* is the most critical food chain for secondary poisoning in case of Cd and PCP. The risk of secondary poisoning methyl mercury is comparable via the routes *soil* → *worm* → *bird/mammal* and *soil* → *worm* → *bird/mammal* → *bird/beast of prey*. The food chains *soil* → *insect* → *bird* → *bird/beast of prey* are the most critical for the other three compounds (DDT, dieldrin, lindane). However, a higher risk for the selected top predator species as compared to strictly worm-eating birds and mammals is only found in the case of DDT. This can be ascribed to the fact that in the food webs of the selected top predator species, worms and insects only form a small percentage of the first trophic level food types. The transfer of contaminants is negligible in plant leaves. Furthermore, corrected NOECs for worm-eaters are somewhat lower than corrected NOECs for vertebrate-eaters due to the lower caloric content of earthworms as compared to birds and mammals.

It seems valid to use the earthworm pathway for screening the risk of secondary poisoning to birds and mammals. Thus in the first place attention can be focused to the route *soil* → *worm* → *bird and mammal*. For persistent and highly lipophilic compounds attention has to be paid to top predators. Then the routes *soil* → *worms* → *birds* → *top predators*, and *soil* → *insects* → *birds* → *top predators*, especially birds of prey should be considered. In recent years, for the derivation of quality objectives for secondary poisoning attention was restricted to the worm-eaters. Model calculations justify this choice for the greater part. An additional advantage is that QSARs for bioconcentration in worms are available for many chemicals. A lack of bioaccumulation data for invertebrate and vertebrate species seriously hampers the derivation of MPCs based on exposure of top predators through comprehensive food webs.

The variation among the selected species with respect to the MPC of the chemicals is high. The lowest MPCs of DDT and dieldrin are found for the Sparrowhawk, while the Little Owl is the most critical species for the other four selected compounds. In case only vertebrate-eating species are considered, the Sparrowhawk is the most critical species for DDT, dieldrin, lindane and cadmium, whereas the Barn Owl is the most critical species for methyl mercury and PCP. The almost exclusively herbivorous mammal-eating birds of prey like Kestrel and Long-eared Owl are among the least exposed species for five of the six selected chemicals. The same applies to the mainly

herbivorous bird-eating Goshawk in case of PCP and cadmium. The Badger is at higher risk to toxic contaminant concentrations in the food than the Weasel, especially for cadmium. The difference in the calculated contaminant-MPCs between the two species can be ascribed to the fact that the Badger is omnivorous, while the Weasel restricts its diet to vertebrates.

The Sparrowhawk and the Barn Owl may be indicated as appropriate bird of prey species for monitoring the risk of secondary poisoning, due to the consumption of quantitatively large portions of insectivorous and seed-eating birds, and insectivorous mammals, respectively.

The model can be adjusted to investigate the risk of secondary poisoning to all kinds of avian and mammalian species. The extra information required is the food choice of these species.

The proposed model can be employed to aquatic ecosystems as well as to terrestrial ecosystems. Risk assessment on food chain contamination for marine birds and mammals and setting of water quality criteria can be based on exposure to chemicals through single food chains (fish-eaters and mussel-eaters) and food webs (species of concern). Information about correction factors (caloric content, assimilation efficiency and metabolic rates) can be found in the appendices of this report. BCFs for several chemicals for fish and mussels are already collected by Romijn et al. (1991a) and Van de Plassche (1994). Toxicity of a number of chemicals to birds and mammals can be retrieved from all three sources.

Recommendations for further research:

1. The developed method should be applied for deriving quality objectives of bioaccumulative chemicals in aquatic environments.
2. More experimental work is needed on the bioconcentration and bioaccumulation of organic compounds in plant seeds and invertebrates.
3. Experiments should be carried out to determine whether there exist differences in sensitivity among both bird and mammal species or taxonomic groups. Determinations of MFO activity can not be used unless they are standardized. Special attention should be given to the Sparrowhawk and the Barn Owl because they seem to be suitable bird of prey species for monitoring strongly bioaccumulative compounds.

4. Risk assessment with this model may be improved by adding estimation of accumulation of contaminants within target organs and comparing with critical organ concentrations.

Elbers and Traas (1993) and Gorree and Tamis (1993) used toxicological data based on critical organ/tissue concentrations in the BIOMAG model. However in general these data are scarce. For some major environmental contaminants, including dieldrin, DDE and mercury, minimum critical levels for reproductive effects in eggs, and for acute effects on brain and liver of raptors are available (Noble and Elliot, 1990; Peakall et al., 1990; Newton et al., 1992). In addition toxicokinetic information is necessary.

5. It should be tried to include fat mobilization in more extreme times (reproductive period, migration, cold periods) in a more physiological model.
6. An uncertainty analysis of the model should be carried out.

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