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Mapping the Potentially Affected Fraction of Avian and Mammalian Target Species in the National Ecological Network

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PREFACE

KEES program

The work reported here is part of the RIVM research program "Kartering Ecotoxische Effecten Stoffen" (KEES; Geographic Representation of Ecotoxicological Effects of Substances). The KEES program comprises a number of related projects, amongst others:

1. Calculation of toxic stress on generic sets of organisms
2. Calculation of toxic stress on target species
3. Indicator toxic stress
4. Measurement of toxic potency of fresh waters in The Netherlands

These four projects have now produced four technical reports, describing how "toxic stress on ecosystems" can be calculated or measured. The first two projects start from measured concentrations of chemicals in soil and water, and calculate toxic stress for (a limited number of) chemicals for which measured concentrations are available. Project 1 focuses at the potential for toxic effects on all possible species (the generic set of species), disregarding the actual occurrence of species at the locations for which toxic stress is calculated. In contrast, project 2 focuses at the species that are thought to occur naturally at the locations of study (sets of target species). Project 3 contrasts to the first two in that it starts from model-calculated concentrations. This way, toxic stress can be calculated for a larger group of chemicals for which emissions and physical-chemical properties are available, but for which measured concentrations are scarce or absent. In project 4 the toxicity of natural waters is assessed by experimental means. Contaminants are concentrated and tested toxicologically. The number of times a given sample needs to be concentrated before toxic effects are observed is used as a measure of the toxic potency of the water sampled.

Potentially Affected Fraction

The four projects have in common that they use the concept of Potentially Affected Fraction of species (PAF). Projects 1-3 calculate toxic stress from known concentrations of chemicals in the environment and laboratory-measured toxicities of these chemicals. This is done for pre-defined sets of chemicals. Project 4 measures the toxicity of water. This is done for unknown mixtures of chemicals. In each case the results are reported in terms of the fraction of species for which the concentration in the environment exceeds the No Observed Effect Concentration (NOEC). We define this fraction as the Potentially Affected Fraction of species (PAF). PAF expresses a potential for adverse effects; no indication is given of the sort of effect or the extent of it.

The PAF concept can best be described as the inverse operation of the well-known "Van Straalen procedure" for deriving "safe" concentrations in the environment. Van Straalen's procedure uses the cumulative NOEC distribution to find the exposure concentration at which a

given fraction (usually 5%) of the species suffers from "above-NOEC exposure". Given a concentration in the environment, the PAF concept finds --using the same NOEC distribution-- the fraction of species that is exposed above NOEC. Both ways of use of the NOEC distribution have been mentioned by Van Straalen (1990), who has pointed out that finding a safe concentration ought to be regarded as "forward use"; reading potentially affected fractions is "inverse use".
At the start of this work, there were --to the best of our knowledge-- no reports of PAF-like procedures of assessment of toxic stress on ecosystems. The potential value of "forward use" of Van Straalen's procedure to yield PAF was first mentioned to us in 1989 by our colleagues T. Aldenberg and W. Slooff in 1989, during the preparation of the "Streven naar waarden" document. Recently, while working on this program, we learned that similar work had been started at RIKZ (Kater and Lefèvre, 1996[1]) and RIZA (Knoben and Beek, 1997).

Goal of the KEES program

The long-term goal of the KEES program is to quantitatively relate concentrations of toxic substances in the environment to species diversity of ecosystems. Toxic stress to ecosystems will eventually be reported as the fraction of species with (too) low probability to occur or survive, given the exposure to toxic substances. Trends of toxic stress in space and/or time can be used to evaluate or develop environmental management policies. Quantification of toxic stress in terms of species diversity will allow comparison of toxic stress with other forms of stress to ecosystems (dehydration, acidification, eutrophication, etc.), for which presently probabilities of occurrence are reported.
The KEES program thus aims at providing rational support for environmental decision making. The results are meant to be used also in the annual RIVM reports on the State of the Environment, and in the four-yearly National Environmental Outlooks.

Present work

The long-term goal of relating concentrations to species diversity is not within immediate reach. The realistic short-term objective of the four above-mentioned projects is to deliver operational procedures to compute and measure Potentially Affected Fractions of species as an indication of toxic stress. It is assumed that spatial and temporal trends in PAF are indicative of trends in toxic effects on ecosystems. However, the indicative value of PAF, its relationship with species diversity, and its potential for environmental decision making are beyond the scope of the present work. The scope is limited to the technical aspects of PAF-assessment.

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[1] Perhaps somewhat confusing in this context, these authors name their risk assessment reading of the NOEC distribution "inverse Van Staalen procedure"!

Future work

On-going work in the KEES program addresses the above-mentioned left-outs. Taking the technical status of the PAF concept as it is reported here as a starting point, the following items are investigated

- Comparison and analysis of the results of the four types of toxic stress assessment. What are the differences and similarities; can this be explained/understood? Is adjustment of the methods necessary/possible?
- Relationship of the Potentially Affected Fraction with ecosystem health. Is there an understandable and predictable relationship between PAF and species diversity? Are the results of PAF assessment useful starting points for assessing the extent of ecosystem damage?
- The possibilities of using PAF as toxic stress indicator in environmental management practice. Do maps of toxic stress provide a meaningful basis for focusing on hot spots? Are trends of toxic stress in time meaningful as indicators of progress in environmental management policy?

TABLE OF CONTENTS

SAMENVATTING

Dit rapport is de tweede in een serie waarin de resultaten van het RIVM onderzoeksprogramma "Kartering Ecotoxicologische Effecten Stoffen" worden gepresenteerd. In dit rapport wordt een methode beschreven waarmee de Potentieel Aangetaste Fractie (PAF) voor doelsoorten (vogels en zoogdieren) behorende bij de natuurdoeltypen van de ecologische hoofdstructuur kan worden berekend. De PAF is de fractie van de doelsoorten die blootgesteld is boven de No Effect Concentratie (NEC) van de doelsoorten. De resultaten worden tevens weergegeven in PAF-kaarten voor Nederland, op basis van achtergrondgehalten, totale concentraties en de anthropogene bijdrage. De methode wordt verduidelijkt aan de hand van drie zware metalen: cadmium, koper en zink.

De PAF's voor het achtergrondgehalte van cadmium zijn tamelijk hoog (>0.1) in West- en Noord-Nederland en relatief laag in de zandstreken in het oosten, midden en zuiden van Nederland. Uitgaande van het totale gehalte van cadmium in de bodem blijkt dat het grootste deel van Nederland PAF's heeft van meer dan 10%, met uitzondering van de duinen in Noord-Holland en de Waddenzee, de Waddenzee zelf en sommige delen van Drenthe. De anthropogene bijdrage is met name hoog in de zandgebieden en speciaal in de Kempen (PAF-waarden van meer dan 50%).

De PAF's voor het achtergrondgehalte van koper zijn relatief hoog in de duinen en langs de grote rivieren. De PAF's voor het totale gehalte aan koper zijn bijna even hoog als die voor het achtergrondgehalte. Hierdoor worden dan ook geen hoge PAF-waarden gevonden voor de anthropogene bijdrage (alle PAF's kleiner dan 10% en de meeste kleiner dan 1%).

De PAF's voor het achtergrondgehalte van zink zijn relatief hoog in the veengebieden van West- en Noord-Nederland en langs de grote rivieren. De PAF's voor het totale gehalte zijn gemiddeld iets hoger dan die voor het achtergrondgehalte in de veengebieden in West Nederland en hetzelfde in de overige gebieden van Nederland. Hierdoor worden slechts in de westelijke veengebieden hogere PAF's gevonden (tussen 10 en 25%) voor de anthropogene bijdrage.

De uitkomsten van het model geven alleen aan welk percentage van de doelsoorten blootgesteld worden aan concentraties hoger dan de NEC van de doelsoorten. Het model geeft geen inschatting van de grootte of hoogte van het effect. In het algemeen worden in het model gemiddelde waarden gebruikt voor de berekeningen (bioconcentratiefactor en dieet) en slechts in enkele gevallen een min of meer worst case benadering (metabole omzetting en toxiciteit). De vertaling van de toxiciteitsparameters naar effecten op de populaties van doelsoorten vindt nog niet plaats, omdat er geen populatiemodellen zijn gebruikt. Daarnaast is het nog niet geheel duidelijk of de hier toegepaste modellen ook geschikt zijn voor essentiële metalen.

Desalniettemin kan het niet worden uitgesloten dat significante stress optreedt bij de hoogste PAF-waarden. Validatie van de resultaten wordt wenselijk geacht.

SUMMARY

This report is the second one in a series in which the results of the RIVM research program "Kartering Ecotoxische Effecten Stoffen" (Geographic Representation of Ecotoxicological Effects of Substances) will be presented. In this report a method is presented for calculating the Potentially Affected Fractions (PAFs) of mammalian and avian target species of the different nature target types of the national ecological network. The PAF is the fraction of species for which the concentration of a certain compound in the environment exceeds their No Effect Concentration (NEC). In addition, a method is provided for mapping the results for the Netherlands. The method is applied for cadmium, zinc and copper. The PAFs for background concentrations of cadmium are already quite high in the western and northern parts of the Netherlands and are relatively low in the more sandy soils of the Netherlands. Based on total concentration for cadmium the PAF is greater than 0.1 in most parts of the Netherlands, except in the dunes of Noord-Holland and the isles of the Waddensea, the Waddensea itself and some parts of Drenthe. High anthropogenic PAFs for cadmium can be found on the sandy soils of the Netherlands, but especially in the Kempen. The PAFs for background concentrations of copper are relative high in the dunes and along the greater rivers. The PAFs for total concentrations of copper are just slightly higher than for the background concentration. Therefore, the anthropogenic PAFs for copper are all low (<0.1). The PAFs for the background concentrations of zinc are relative high in the peat areas of the western and northern Netherlands and along the greater rivers. The PAFs for the total concentrations of zinc are on average slightly higher in the western peat areas of the Netherlands. Therefore, elevated PAFs for the anthropogenic contribution are only found in the western peat areas. The model applied in this report provides the number of target species (expressed in percentages) that are exposed at concentration above the NECs of the target species. The model does not give an estimation of the extent of the effects that could be expected in the field (e.g. a 45% effect on reproduction). In the model, median estimates were used for bioconcentration factors and diet composition. More conservative approaches have been used for the metabolic rate and the toxicity. The translation of the toxicological endpoints to effects on a population level is still not adequate, since no population models were used. It can be expected that significant toxic stress occurs at the highest PAFs, but validation of these results is needed.

1. INTRODUCTION

In 1990 the Dutch Ministry of Agriculture, Nature Management and Fisheries issued the Nature Policy Plan. The central point was the establishment of a national ecological network, consisting of core areas (existing nature reserves), natural development areas and ecological corridors. In 1995 the Handbook of nature target types in The Netherlands was published (Bal et al., 1995). This handbook provides the methodological basis for the realisation of the national ecological network by means of a comprehensive set of nature target types. Each of the 132 units in this classification specifies an ecological objective in terms of biotic and abiotic components at a particular scale. In this handbook 657 species from ten taxonomic groups have been selected as target species. The selection is based on an assessment of international significance, knowledge on trends in population size and on national red data lists.

The aim of this report is to provide a method with which the percentage of the target species of a certain nature target types that will be exposed to concentrations higher than the protection level of those species, can be calculated. Data availability limited the method at present to birds and mammals. In addition a method will be provided with which the results can be mapped for The Netherlands. The method will be elucidated with cadmium, zinc and copper as examples.

2. METHOD OVERVIEW

The method used for mapping the potentially affected fraction of target species can be subdivided into several components (Figure 1). Below, a brief description of each component is given. Components will be described in full in subsequent chapters.

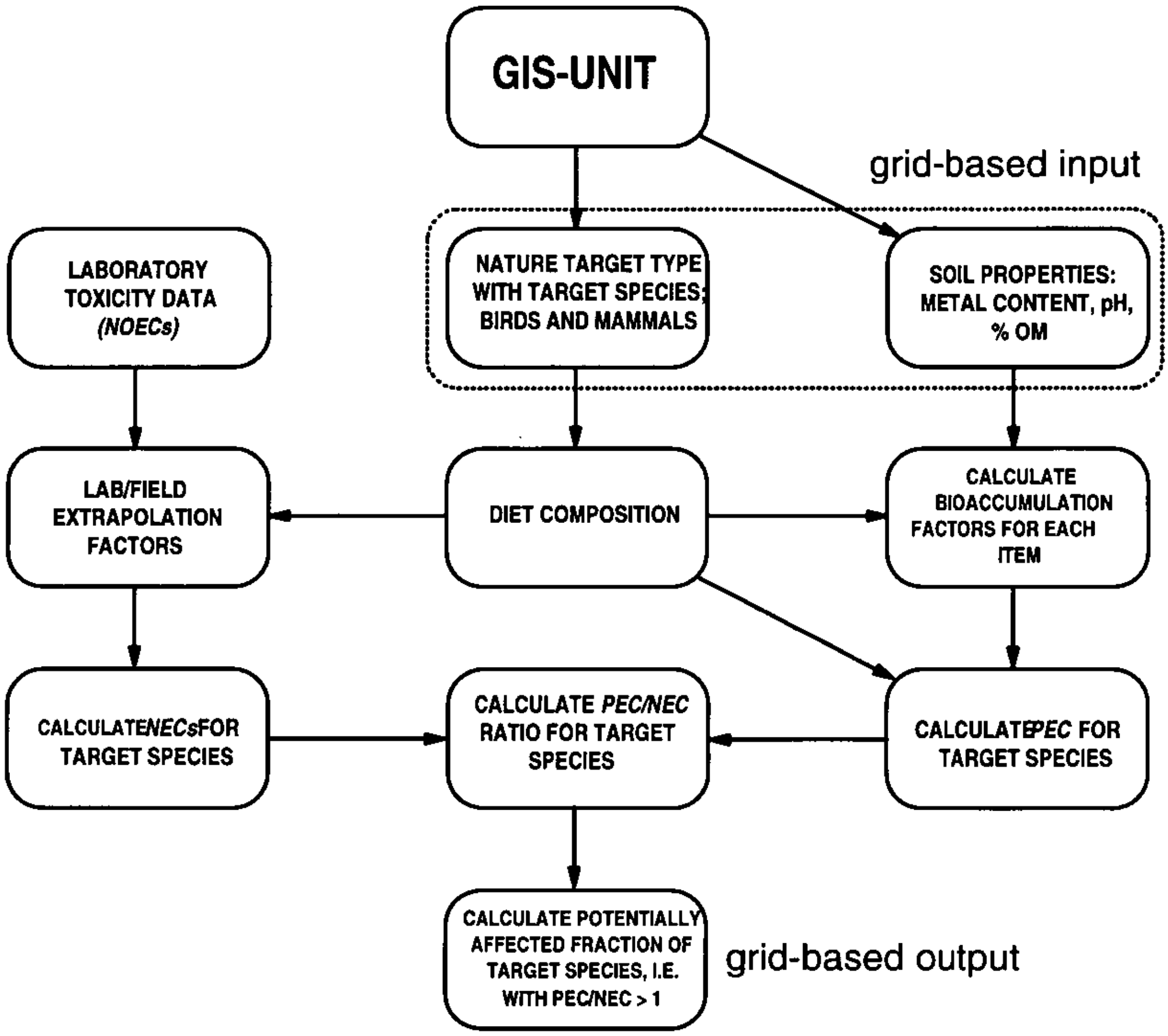


Figure 1: Flow-chart for calculating the Potentially Affected Fraction (PAF) for target species at a specific location. NEC = no-effect concentration, PEC = predicted environmental concentration.

2.1 Geographical data

The potential area designated for the National Ecological Network (NEN) in The Netherlands is shown in Figure 2. Each unit of the map represents a certain ecosystem (as defined by the nature target type (Bal et al., 1995)) considered dominant for that location. The nature target type determines which birds and mammals are indicators for a specific location.

The Laboratory for Soil and Groundwater provided maps for soil properties such as metal content (Cu, Cd, Zn), pH and organic matter content (Klepper and Van der Meent, 1997). These data are used as input for calculations. From these data, metal concentrations associated with the mineral fraction of the soil (background concentrations) were calculated by Klepper and Van de Meent (1997). These concentrations are used for calculating the potential affected fraction.

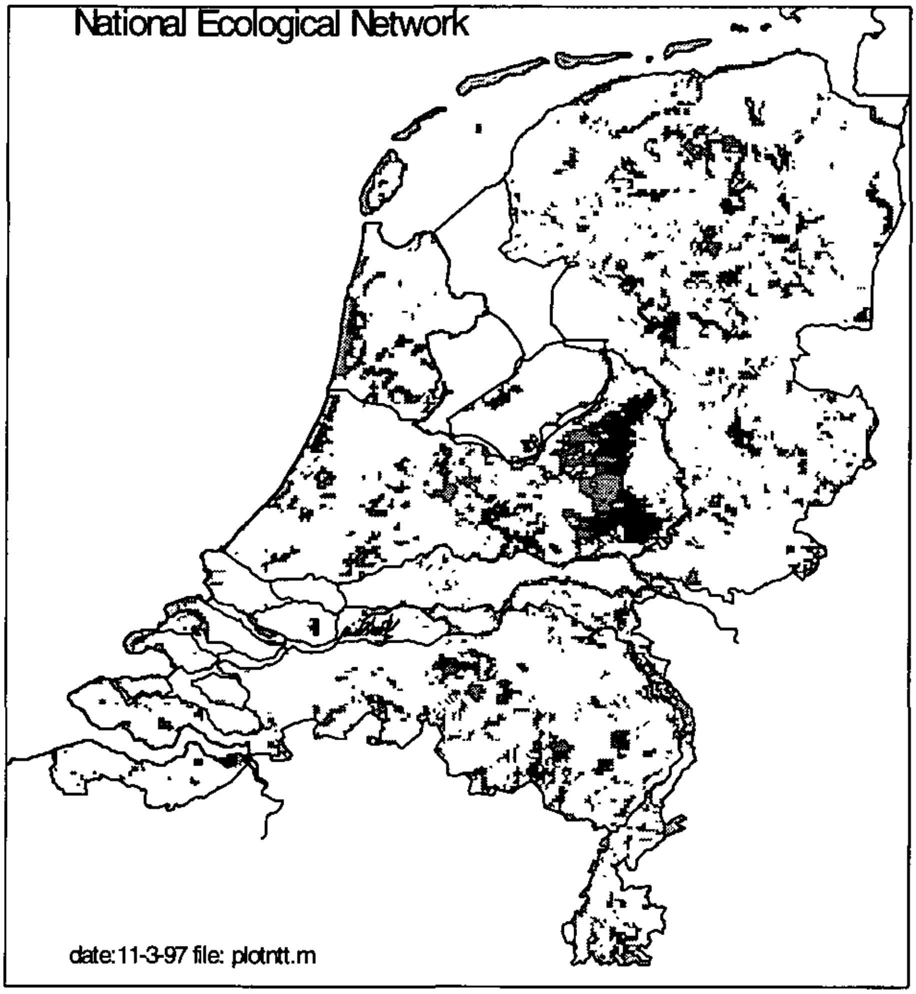


Figure 2: Area designated for the National Ecological Network in The Netherlands (courtesy of SC-DLO, 1996).

2.2 Calculation of NEC

The left side of Figure 1 shows the steps needed to calculate the No Effect Concentration (NEC) for target species. In this paragraph these steps are briefly discussed.

2.2.1 Laboratory toxicity data

In order to estimate the risk for a target species (in this case birds and mammals), the exposure concentration must be compared to a No-Effect Concentration (NEC) for that particular species. Since toxicity data are extremely scarce for target species (Posthuma et al., 1995), laboratory toxicity data on rats, mice, rabbits, quail etc. are used to estimate wildlife toxicity. For distinguishing extrapolated No Observed Effect Concentration (NOEC) for a target species from the NOECs of test species, the former one will be called the NEC.

2.2.2 Lab to field extrapolation factors

In order to extrapolate laboratory toxicity data to the field (i.e. NOECs to NECs), several factors influencing the exposure and sensitivity of target species have to be considered. Of the factors proposed by Luttik et al. in 1992, those that can be quantified are taken into account (see for species sensitivity and pollutant assimilation efficiency Jongbloed et al. (1994) and Traas et al. (1996)):

- Metabolic rate extrapolation factor, constant for all target species
- Caloric content of food, depending on diet composition of target species
- Food assimilation efficiency, depending on diet composition of target species

2.2.3 NEC of target species

The No Effect Concentrations (NECs) of target species are estimated from laboratory toxicity data using lab to field extrapolation factors. The laboratory toxicity data are assumed to derive from a log-logistic distribution. Due to the fact that the extrapolation factors are the same for all individual NOECs, the factors only influence the location (i.e. shift position on the log concentration axis), mot the variance of the data. The extrapolation factors only influence the location of the data, not the variance of the data. The NEC for target species is related to specific percentiles of the resulting log-logistic distribution. As an median estimate of the NEC the median values (50th percentile) of the resulting log-logistic distribution of (extrapolated) toxicity data is used.

2.3 Calculation of PEC for target species

The right side of Figure 1 shows the steps needed to calculate the Predicted Environmental concentration (PEC) for target species.

2.3.1 Target species and their diet

Within the ecological network, 80 species of birds and mammals were chosen as ecological quality indicators (Appendix 1). Each unit of the National Ecological Network is assigned its dominant nature target type with associated species (Bal et al., 1995). For all birds and mammals, the risk of food chain poisoning is calculated using annually-averaged diets (long term chronic exposure). The diet of the target species is also needed to calculate the extrapolation factors for caloric content and food assimilation efficiency.

2.3.2 Bioaccumulation data

In order to calculate the concentration of metals in dietary food items, bioaccumulation factors are needed. For each diet component, bioaccumulation factors were collected from the literature (Jongbloed et al. 1994, 1996). Median bioaccumulation factors were used for the calculations. For earthworms, a regression equation is used to estimate metal concentrations taking the effect of pH on bioconcentration into account (Verhallen and Ma, 1997).

2.3.3 PEC for target species

The potential environmental concentration (PEC) for exposure of birds and mammals is the average diet concentration, through which these animals are exposed to the toxicant. By calculating the weighted average of the concentration of all diet items for target species, the average food concentration for all relevant target species is calculated for a specific location.

2.4 Calculation of PEC/NEC ratio for target species

For each target species, the potential average concentration in the diet (PEC) can now be compared to the No Effect Concentration for the diet. When the PEC/NEC ratio is larger than unity, the target species is considered at risk. When the ratio is smaller than unity, no risk is considered present.

2.5 Calculation of the Potentially Affected Fraction (PAF) for target species

The PEC/NEC ratio has been calculated for all bird and mammal target species in each geographical unit of the national ecological network. The fraction of the species that is potentially affected, is the number of target species with PEC/NEC ratio larger than unity, divided by the total number of bird and mammal target species for a specific location.

It has been proposed by Klepper and Van de Meent (1997) that heavy metals (Cd, Cu and Zn) associated with the mineral fraction of the soil are considered background values not to be taken into account in the risk assessment. Therefore, the whole procedure described above is performed twice. The first time, Potentially Affected Fractions are calculated for background concentrations in soil, and the second time for the total concentrations in soil. In this way, the anthropogenic heavy metal pollution is distinguished from the naturally occurring background concentrations.

3. MATERIALS AND METHODS

3.1 Geographical data

Geographically explicit data are used to determine the type of ecosystem (nature target types) with their associated target species. Heavy metal concentrations, background concentrations and soil parameters modifying the uptake of metals by organisms are also needed in geographical units.

3.1.1 Nature target types

Bal et al. (1995) provided a set of nature target types. Each of the 132 units in this classification specifies an ecological objective in terms of biotic and abiotic components. In The Netherlands 9 physical-geographical regions are distinguished:
hilly country (heuvelland), higher sandy soils (hogere zandgronden), river clay area (rivierengebied), peat-bog area (laagveengebied), marine clay area (zeekleigebied), dunes (duinen), former sea-arms (afgesloten zeearmen) , tidal area (getijdengebied), and North Sea (Noordzee).
The map of nature target types was made available by the Staring Centre and has a resolution of 1 by 1 km. Each unit of the National Ecological Network (Figure 2) is assigned a dominant nature target type.

3.1.2 Heavy metal concentrations in soils

Heavy metal concentrations in the top soil (0-10 cm) were calculated from extrapolation of a large data base of measured heavy metal concentrations. Known relationships for sorption of heavy metals to soil and historic loading records were used to calibrate a model for extrapolation of measured concentrations to concentration maps for heavy metals in The Netherlands. These maps were made available as a 650 by 560 grid with a gridsize of 0.5 by 0.5 km (see Klepper and Van der Meent, 1997).
In addition heavy metal concentrations were measured in reference locations. From these data, regressions were made on the amount of metals naturally associated with the mineral fraction and the organic fraction of the soil (Lexmond en Edelman, 1992). These regressions were used to estimate the background concentrations for the metals Cd, Cu, Zn and Pb in The Netherlands (Klepper and Van de Meent, 1997). For the calculation of the PAF for target species, the calculated average background concentration was used.

3.2 Calculation of NEC

3.2.1 Laboratory toxicity data

In the next three tables the toxicity data (NOECs) for birds and mammals for Cd, Zn and Cu are presented. These are the converted data as given by Jongbloed et al. (1996). For cadmium 5 NOECs for birds and 7 NOECs (5 species) for mammals could be found. For copper and zinc less studies were available; copper 4 mammals and 1 bird and for zinc 1 mammal and 1 bird. For copper and zinc a combined set of toxicity data (mammals + birds) were used for the calculation of NEC values. For cadmium birds and mammals were treated separately.

Table 1 Cadmium toxicity data for birds

Species	Endpoint	Exposure period (days)	NOEC mg/kg food
Meleagris gallopavo	growth	14	0.2
Anas platyrhynchos	reproduction	90	1.6
Gallus domesticus	mortality/ reproduction	36	12
Coturnix c. japonica	growth	42	38
Streptopelia risoria	reproduction	150	1.9

Table 2 Cadmium toxicity data for mammals

Species	Endpoint	Exposure period (days)	NOEC mg/kg food
Macaca mulatta	growth	1095	3
Ovis amon aries	growth	191	15
Rattus norvegicus	growth	730	10
Rattus norvegicus	growth	90	42
Bos primigenius taurus	growth	84	40
Sus scrofa domesticus	growth	153	40
Sus scrofa domesticus	growth	42	50

Table 3 Toxicity data for zinc and copper (birds and mammals)

	Species	NOEC mg/kg food	
Copper:			
Mammals	Ovis amon aries	7	mg/kg food
	Mus musculus	40	mg/kg food
	Sus scrofa domesticus	250	mg/kg food
	Rattus norvegicus	265	mg/kg food
Birds	Gallus domesticus	150	mg/kg food
Zinc:			
Mammals	Ovis amon aries	150	mg/kg food
Birds	Gallus domesticus	1000	mg/kg food

In Figure 3 and Figure 4 the calculated logistic distribution curves for cadmium and the underlying data are graphically presented. The α and β values of these curves are calculated with the ETX-program of Aldenberg (1993).

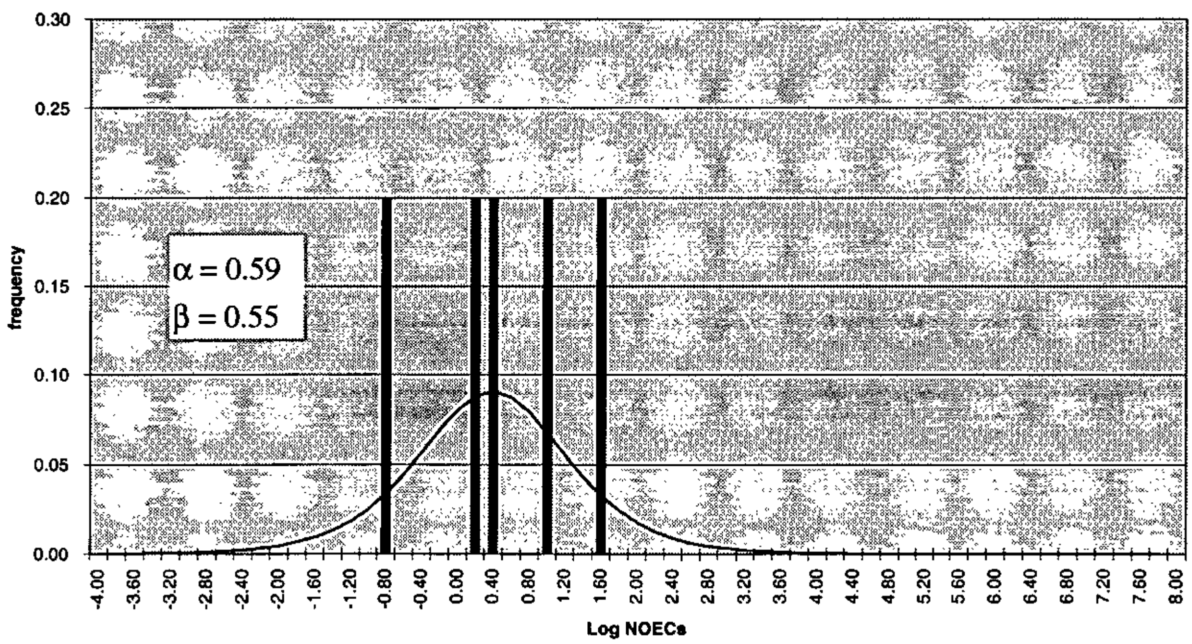


Figure 3: Logistic distribution of cadmium NOECs for birds.

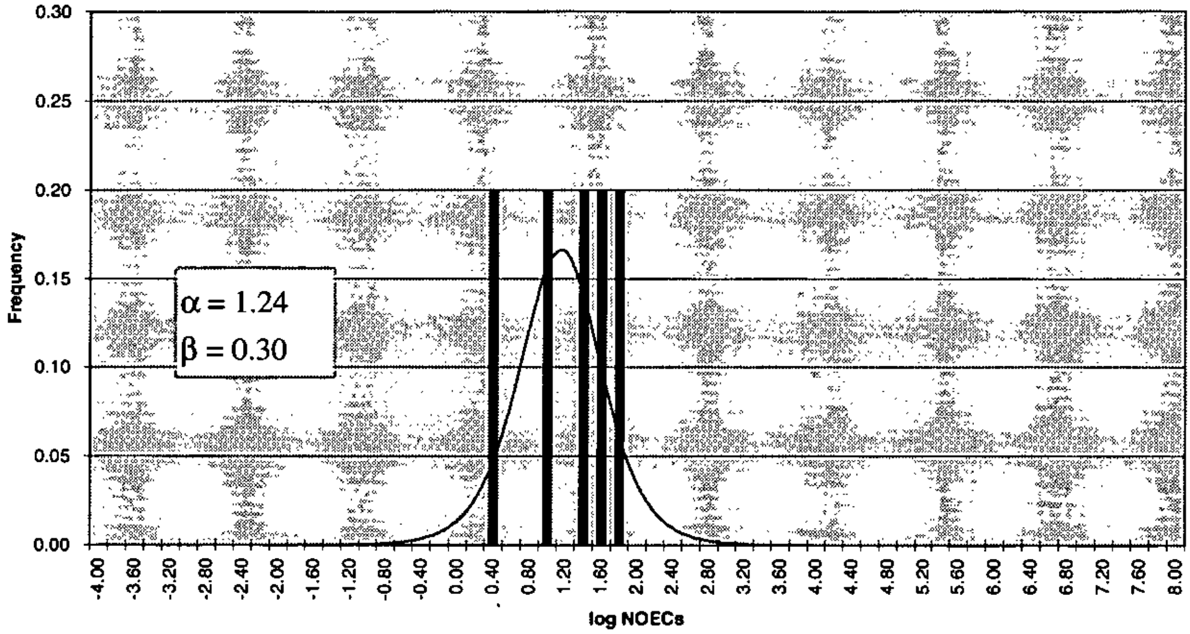


Figure 4: Logistic distribution of cadmium NOECs for mammals.

3.2.2 Lab to field extrapolation factors

When extrapolating toxicity data derived from laboratory test with bird and mammals to target species in the field, several factors concerning exposure and sensitivity should be considered (Luttik et al., 1992; EPA, 1993 and Health Council, 1993).

- metabolic rate
- caloric content
- food assimilation efficiency
- pollutant assimilation efficiency
- species sensitivity

The rationale behind these correction factors is based on three assumptions. The first assumption is that total dose received should be equal for test species and target species of equal weight under equal circumstances. The second assumption is that free-roaming animals use more energy than caged test species and therefore receive a larger dose. The third assumption is that the sensitivity of target species could be fundamentally different from the test species used in toxicity testing.

For the first three factors it has been demonstrated by Traas et al. (1996) that the difference between target species and test species could be substantial. For these three factors a correction method will be incorporated in the model.

The literature on the fourth factor, pollutant assimilation efficiency, is very sparse and often not related to target species (particularly for birds and mammals). Due to the limited and contradictory information on organic compounds it was decided by Traas et al. (1996) not to correct for the assimilation efficiency of such compounds. From the information on cadmium and methyl mercury it could be concluded that corrections for the assimilation of Cd and MeHg for mammals were not necessary (no information on birds).

In Jongbloed et al. (1994) and Traas et al. (1996) arguments are presented that showed that the assumed difference by Walker et al. (19??) in species sensitivity (seabirds versus non-seabirds and raptors versus non-raptors) are not valid. It was demonstrated that the standard test species are not less sensitive than other species. Organophosphorus compounds can be an exception because the available information (LD50 values) shows a tendency for raptorial birds to be more sensitive for these compounds than other birds.

3.2.2.1 Metabolic rate

Three metabolic rates most commonly used are:

1) *Basal Metabolic Rate* (BMR) - the metabolic rate within the thermoneutral zone of ambient temperatures when the animal is at rest and in a postabsorptive state.

2) *Existence Metabolic Rate* (EMR) - the metabolic rate necessary for an animal to maintain itself in captivity without a change in body weight.

3) *Field Metabolic Rate* (FMR) - the total daily energy requirement for an animal in the wild.

It would have been convenient to search in the extensive literature for the best estimate of FMR and EMR and calculate an FMR/EMR ratio. However, there are some difficulties in doing this. Several methods are used for measuring metabolic rates. The BMR is generally measured by assessing the oxygen consumption and carbon dioxide output in metabolic chambers. The EMR is sometimes measured in terms of oxygen consumption, but more often it is determined by calculating the energy content of the food digested, reduced by the energy content of the excreta. FMRs are mostly measured using doubly labelled water (DLW) to measure the carbon dioxide production. There are indications that the estimates of energy metabolism by oxygen consumption may differ from DLW measurements by up to 50% in birds and mammals (Nagy, 1987).

Many allometric equations relate to small groups of birds and mammals, sometimes with the aim of calculating the influence of other parameters on the BMR - body weight (BW) relationship. Regressions given in the literature are rarely accompanied by confidence intervals. Hayssen and Lacey (1985) found that 21% of the measured values of BMR in mammals

deviated by more than 50% from the calculated equation. An additional problem is that allometric equations for EMR are scarce (especially for mammals).
But suppose that the following allometric equations log(EMR) = 0.658+0.664 * log(BW) (Ellis, 1984) and log(FMR) = 0.982 + 0.661 * log(BW) (Williams, 1988) are the most appropriate ones for the EMR and the FMR (see 2 lines in Figure 5A). By knowing the body weight of the target species and the test species the extrapolation of the EMR to FMR (lab to field) would be easy. However in practice the situation is very often more complicated. There is not one single body weight but a range of body weights (see figure 5B). The body weight of the test species is often not given in the article (older literature), or the test was started with juvenile birds and ended when the birds were adult (the body weight range for a Japanese quail in this case would be from several grams to 100 grams). For some species it would be more appropriate to make an extrapolation for females as well as males. The example in figure 5B is the situation of extrapolating from chickens (Gallus domesticus) to marsh harriers (Circus aeruginosus): target species 1 are the females and target species 2 are the males.
Because of these problems it was decided to use a different approach to determine the difference between EMR and FMR, namely by calculating ratios for individual species. Single EMR values have been measured for many birds and mammals and are about 1.5 to 2 times the BMR (Kirkwood and Bennett, 1992). By comparing the 68 paired BMR and EMR values for birds and mammals from the database of Kendeigh et al. (1977) an EMR/BMR ratio of 1.45 ± 0.57 could be calculated. Daan et al. (1990) calculated a FMR/BMR ratio for individual species of 3.57 ± 0.97. The lowest value they found was 1.87, and the highest 5.59. Koteja (1991) listed paired FMRs and BMRs for 52 birds and mammals. The FMR/BMR ratio varies from 1.6 to 6.6, the average value being 3.77. Unfortunately these animals are not a representative sample of all birds and mammals. Bryant and Tatner (1991) determined the FMR/BMR ratio of 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 for a bird or mammal is not constant throughout the year. The FMR for periods of energy-demanding conditions (e.g. breeding season or low temperature) is higher than the FMR under more normal conditions. Peterson et al. (1990) suggested that there is a certain level that can be sustained during a relative long period, without using stored reserves (sustained metabolic rate; SusMR). The existence of a SusMR may be explained by the concept that there must be upper limits to both the capacity of the digestive tract to process food and the cellular capacity. The 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 episodes of sustained metabolic rates.
Kirkwood (1983) searched for FMR data for animals under energy-demanding conditions such as hard exercise, rapid growth, breeding, lactation or low temperatures. The maximum energy expenditure was between 3 and 6 times BMR. Data from birds, humans and other mammals had

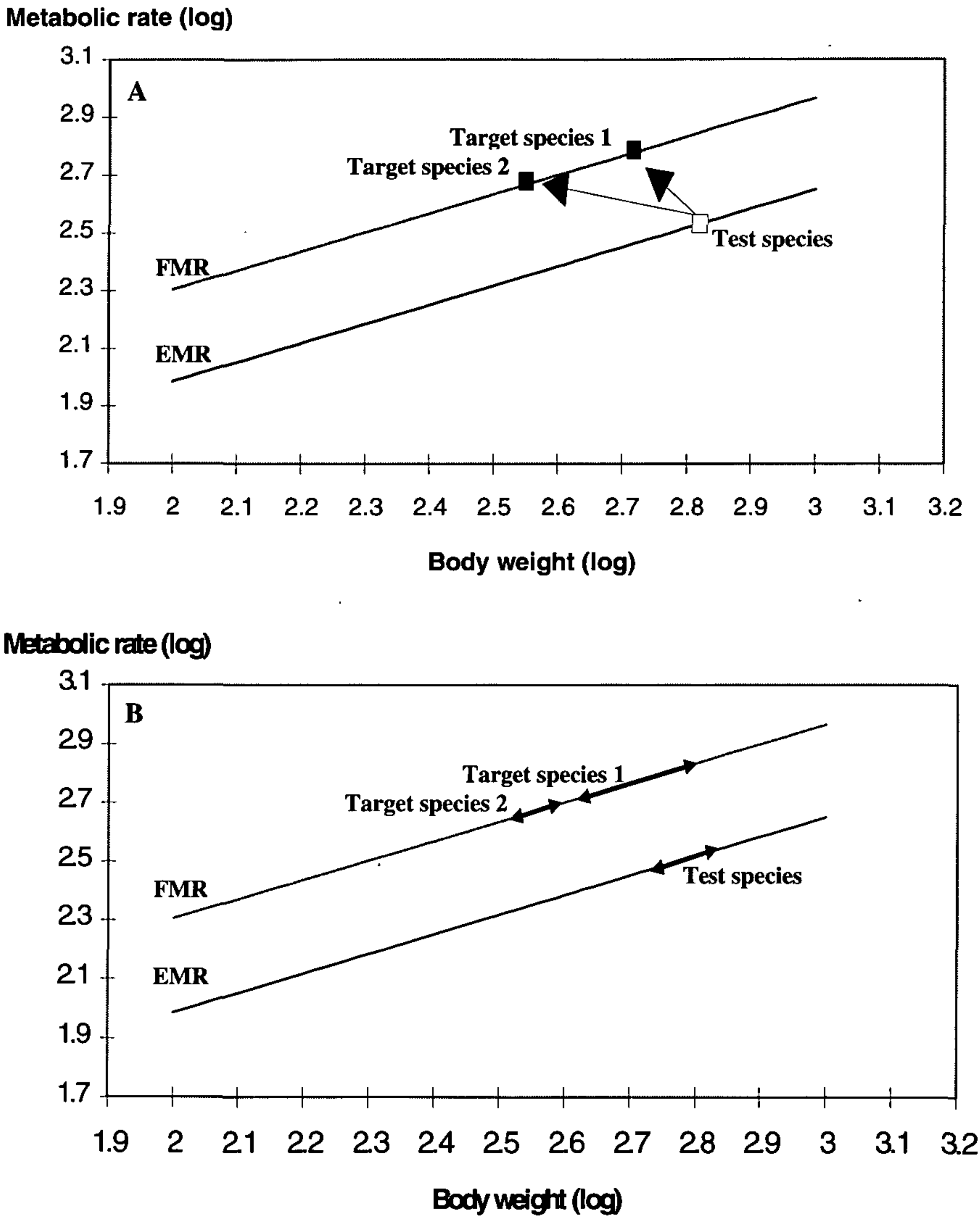


Figure 5: Relationship between existing metabolic rate (EMR) and field metabolic rate (FMR); figure A for single body weights (e.g. mean) and figure B for ranges of body weights.

SusMR/BMR ratios mostly of 1.5 to 5 (Peterson et al., 1990). Outliers were always lower than 7. A SusMR of 6 times BMR seems to be a realistic value.

By using the fixed ratios of ± 1.5 and ± 3 for EMR/BMR and FMR/BMR relationships, respectively it was decided to use a correction factor of 0.4 for differences between laboratory and field in metabolic rates for normal circumstances. In case of more extreme energy demanding periods a correction factor of 0.25 can be used for the ratio EMR/SusMR.

3.2.2.2 Caloric content

The caloric content of food largely determines the amount of food that has to be consumed in order to meet the nutritional demand of an animal. Birds and mammals tested in the laboratory are generally fed with commercial mixtures (fodder) with a relative high energy content, based on wet weight (see Table 4). It can be expected that laboratory animals consume less food on a wet weight basis compared to animals in the field. In general the natural food items have a lower caloric content (CC) than fodder. Only the CC of seeds is higher than the CC of fodder. The CC of mammals and birds as food is approximately a factor 2 smaller than fodder. When leaves are the main food item the factor between fodder and leaves is approximately 20, which means that a leaf-eating animal has to consume on wet weight basis 20 times more than a test animal. These differences can partly be ascribed to differences in water content (see Table 4).

3.2.2.3 Food assimilation efficiency

The amount of food consumed is also determined by the available energy which can be used by the animals for production and maintenance. This part of the energy, the metabolizable energy (ME), is the gross energy (GE) in an unit of food consumed minus the energy lost in faeces

Table 4: Caloric content (CC in kJ/g ww) ,water content (%H_2O) of several types of food and the correction factor (CF) for extrapolating from laboratory to field conditions (after Jongbloed et al, 1994).

Food type	CC mean	SD	n	Range	%H_2O mean	CF_{bird}	CF_{mammal}
Fodder birds	13.7	2.8	6	11.3 - 17.4	11	n.a.	n.a.
Fodder mammals	16.8	-	2	11.8 - 22.8	6	n.a.	n.a.
Terrestrial:							
Leaves	0.9	0.7	7	0.3 - 2.3	92	0.07	0.05
Fruit/buds	2.5	1.5	11	1.0 - 5.8	83	0.18	0.15
Seeds	19.9	6.3	4	16.4 - 29.4	10	1.45	1.18
Roots	1.5	0.7	3	0.8 - 2.4	91	0.11	0.09
Earthworms	3.0	0.6	3	2.3 - 3.4	84	0.22	0.18
Snails	3.8	1.3	8	2.1 - 5.6	83	0.28	0.23
Spiders	7.2	No data, as for insect (adult)			70	0.53	0.43
Insects (larvae)	5.2	3.3	8	1.9 - 11.5	77	0.38	0.31
Caterpillars	7.2	No data, as for insect (adult)				0.53	0.43
Insects (adult)	7.2	1.6	10	3.2 - 8.8	66	0.53	0.43
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
Aquatic:							
Snails	3.8	1.3	8	2.1 - 5.6	83	0.28	0.23
Crustaceans	4.4	1.4	12	3.1 - 5.8	67	0.32	0.26
Insect (larvae)	7.2	No data, as for insect (adult)			71	0.53	0.43
Insect (adult)	7.2	No data, as for insect (adult)			71	0.53	0.43
Amphibians	6.2	No data, as for fish				0.45	0.37
Fish	6.2	1.9	58	2.9 - 11.2	75	0.45	0.37

and urine. Assimilation efficiency (AE) equals the ratio ME/GE, or the fraction of GE that can be metabolized.

By assuming that every animal of the same weight needs the same amount of energy the assimilation efficiency dictates the amount of food an animal has to consume. In case the amount of food consumed by a target animal is relative higher than the test animal of the same weight one can expect that the exposure to pollutants is higher too (test results of dietary studies are expressed in mg per kg wet weight food).

The mean assimilation efficiencies of the energy of different types of food are mostly of the same magnitude; 0.67 to 0.78 for birds and 0.79 to 0.87 for mammals (see Table 5 and 6). When leaves are the food item the mean assimilation efficiency is 0.37 and 0.50, for birds and mammals, respectively, which means that leaf eating birds and mammals have to consume approximately twice as much as laboratory animals fed with commercial fodders.

Table 5: Assimilation efficiency (AE) of several types of food for birds and the correction factor (Cf_{birds}) for extrapolating from laboratory to field conditions (after Jongbloed et al, 1994).

Food type	AE	SD	n	Range	CF_{bird}
Fodder birds	0.73	0.096	41	0.42 - 0.92	n.a.
Terrestrial:					
Leaves	0.37	0.075	6	0.30 - 0.51	0.51
Fruits/buds	0.56	0.15	8	0.37 - 0.77	0.76
Seeds (kernels)	0.76	0.13	13	0.47 - 0.91	1.04
Roots	0.76	No data, as seeds			1.04
Earthworms	0.73	No data, as fodder			1.00
Snails	0.81	No data, as squid			1.11
Spiders	0.67	No data, as insects (adult)			0.93
Insects (larvae)	0.78	0.053	8	0.70 - 0.86	1.06
Caterpillars	0.78	No data, as insect (larvae)			1.06
Insects (adults)	0.673	0.101	6	0.56 - 0.82	0.92
Birds	0.749	0.068	8	0.68 - 0.85	1.02
Mammals	0.746	0.072	39	0.61 - 0.94	1.02
Aquatic:					
Snails	0.81	No data, as squid			1.11
Crustaceans	0.78	No data, as insect (larvae)			1.06
Insect (larvae)	0.78	No data, as insect (larvae) above			1.06
Insect (adult)	0.67	No data, as insect (adult) above			0.92
Amphibians	0.78	No data, as fish			1.06
Fish	0.78	0.04	15	0.69 - 0.85	1.06

Table 6: Assimilation efficiency (AE) of several types of food for mammals and the correction factor ($CF_{mammals}$) for extrapolating from laboratory to field conditions (after Jongbloed et al., 1994).

Food type	AE	SD	n	Range	CF_{mammal}
Fodder mammals	0.86	0.13	3	0.71 - 0.95	n.a.
Terrestrial:					
Leaves	0.495	0.168	25	<0.20 - 0.898	0.57
Fruits/buds	0.56	No data, as table for birds			0.65
Seeds	0.895	0.007	2	0.89 - 0.91	1.04
Roots	0.895	No data, as for seeds			1.04
Earthworms	0.86	No data, as for fodder			1.00
Snails	0.81	No data, as table for birds			0.94
Spiders	0.86	No data, as for insect (adult)			0.99
Insect (larvae)	0.86	No data, as for insect (adult)			0.99
Caterpillars	0.86	No data, as for insect (adult)			0.99
Insect (adult)	0.86	0.065	4	0.78 - 0.93	0.99
Birds	0.79	No data, as for mammals			0.92
Mammals	0.79	0.112	6	0.627 - 0.901	0.92
Aquatic:					
Snails	0.86	No data, as for insects (adult)			0.99
Crustaceans	0.86	No data, as for insects (adult)			0.99
Insect (larvae)	0.86	No data, as for insects (adult)			0.99
Insect (adult)	0.86	No data, as for insects (adult)			0.99
Amphibians	0.79	No data, as for mammals			0.92
Fish	0.79	No data, as for mammals			0.92

3.2.3 NEC of target species

Statistical methods are used to extrapolate species sensitivities from a small number of NOECs or LC_{50}s to the sensitivity of other non-tested species. It is generally assumed that the underlying interspecific distribution of species sensitivity follows a log-normal or log-logistic distribution (Aldenberg and Slob, 1993, Wagner and Løkke, 1991). The statistical population from which we will sample is the set of LC50s or NOECs for birds and mammals determined under laboratory conditions. It is assumed that their sensitivity to toxicants can be different under field conditions, for which lab to field extrapolation factors may be applied. After applying these

factors, we have a new set of NECs from which we can calculate the parameters of the log-logistic distribution (Traas et al. 1996).
The extrapolation factors that were found to be important (Traas et al. 1996) can now be used to extrapolate a set of NOEC data from the open literature to birds and mammals in the field. It concerns a limited set of toxicity tests where birds and/or mammals have been exposed to contaminated food (Jongbloed et al., 1994).

NECs for species in the field are calculated with

$$NEC_{field} = NOEC_{lab} \cdot fCC \cdot fFAE \cdot fMR \qquad (1)$$

where NEC_{field} = No Effect Concentration for a target species in the field
$NOEC_{lab}$ = No Observed Effect Concentration of laboratory test species
fCC = extrapolation factor for Caloric Content (CC)
$fFAE$ = extrapolation factor for Food Assimilation Efficiency (FAE)
fMR = extrapolation factor for Metabolic Rate (MR)

The extrapolation factors for caloric content (fCC) and food assimilation efficiency (fFAE) are calculated from a weighted average of the different diet items for the top predator:

$$\frac{\sum_{i=1,n} FAE_i \cdot fDiet_i}{FAE_{ref}} \quad \text{and} \quad \frac{\sum_{i=1,n} CC_i \cdot fDiet_i}{CC_{ref}} \qquad (2)$$

where FAE_i = Food assimilation efficiency of diet item i
CC_i = Caloric content of diet item i
$fDiet_i$ = Fraction of diet of item i
FAE_{ref} = laboratory FAE (equal for birds and mammals)
CC_{ref} = laboratory CC (different for birds and mammals)

This results in a set of NECs for field conditions, corrected for the specific feeding habits of target species. From this set, assuming a log-logistic distribution of species sensitivities, the **median** (50th percentile) NEC estimate was used to represent a specific target species. For each target species, the NEC was calculated in this way. The median estimate of the resulting NEC distribution is chosen as the best estrimate of the sensitivity of an untested species, being neither too protective or too careless. An example calculation for one NOEC from the set of laboratory NOECs is given below.

The diet of a Barn Owl (Tyto alba) consists for 93% of mammals and for 7% of birds.
The caloric content of fodder in bird toxicity studies is 13.7 kJ/g ww and for birds and mammals, 7.9 kJ/g ww and 7.1 kJ/g ww respectively. The food assimilation factor is 0.73 for fodder and 0.75 for birds and mammals as food. The correction factor for the metabolic rate is 0.4.

Applying equations 1 and 2, we get

$$NEC_{BarnOwl} = NOEC_{Testspecies} \cdot \left[(0.93 \cdot 7.1 + 0.07 \cdot 7.9)/13.7\right] \cdot \left[(0.93 \cdot 0.75 + 0.07 \cdot 0.75/0.73)\right] \cdot [0.4]$$

$$= NOEC_{Testspecies} \cdot 0.52 \cdot 1.03 \cdot 0.4 = NOEC_{Testspecies} \cdot 0.21$$

After treating each NOEC from the laboratory in this way, the distribution of field corrected NECs and lab NOECs are both known (Figure 6). Note that due to the fact that the extrapolation factors are the same for all individual NOECs, the factors only influence location (i.e. shift position on the log concentration axis), not the variance of the data.

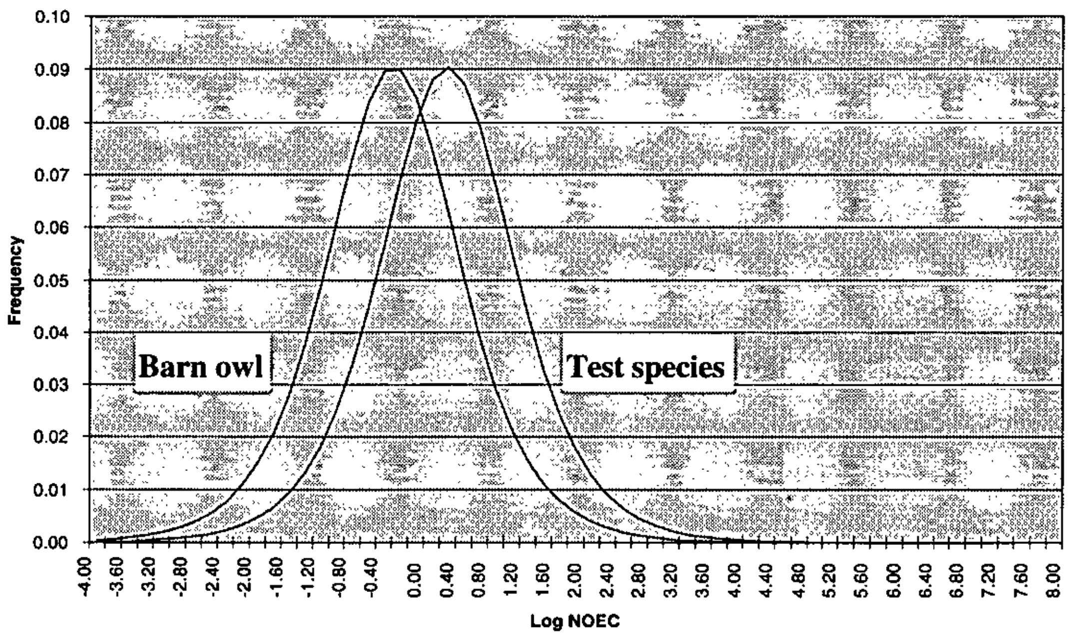


Figure 6: Logistic distribution of the Cd toxicity data (n=5, see table 1 and figure 3) for the test species and a target species (Barn Owl)

3.3 Calculation of PEC

3.3.1 Target species and their diet

3.3.1.1 Target species

657 species from ten taxonomic groups have been selected as target species (Bal et al., 1995). The selection is based on an assessment of international significance, knowledge on trends in population size and on red data lists. The ten taxonomic groups are; higher plants (408 species), mammals (16 species), birds (64 species), reptiles (5 species), amphibians (7 species), butterflies (47 species), dragonflies (20 species), fishes (56 species), crustaceans (24 species), and echinoderms (10 species). The method described in this report is only based on the mammalian and avian species (12% of all target species). At this moment we are not able to incorporate the other species in the model, either due to a lack of information (toxicity and ecological parameters) or we are not able to differentiate between species (the same route of exposure, for instance via ground water or crustaceans via the water).
The average number of species in a nature target type is 23 and ranges between 3 and 57 species (see also table 7).

Table 7: The number of nature target types in the physical-geographical regions of The Netherlands and the average number of species in the nature target types.

Region	---- Number of ---- nature target types	-------- Number of species -------- average	minimum	maximum
Hilly country	19	21	6	34
Higher sandy soils	28	24	6	57
River clay area	19	23	11	50
Peat-bog area	19	24	10	45
Marine clay area	21	23	7	48
Dunes	22	19	3	52
Former sea-arms	15	23	7	44
Tidal areas	6	31	26	33
North Sea	1	9	9	9

3.3.1.2 Diet

Starting point for the research on the diets of the target species was the Handbook of the Birds of Europe, the Middle East and North Africa: the Birds of the Western Palearctic (Cramp et al, 1977 - 1994) and the Handbuch der Säugetiere Europas (Niethammer and Krapp, 1978-1994). In addition the following literature was used: Beck (1995) Fecal analyses of European bat species, Böhme (1984) Handbuch der Reptilien und Amphibien Europas, Glutz von Blotzheim and Bauer (1966-1993) Handbuch der Vögel Mitteleuropas. The results are presented in Appendix 1.

3.3.2 Bioaccumulation data

3.3.2.1 Fixed bioaccumulation factors

The basic data for cadmium in table 8 were collected by Jongbloed et al. (1994) and Romijn et al. (1993 and 1994) and in addition an on-line research was carried out in 1996 to update these data. The data for zinc and copper were collected as described in Jongbloed et al. (1994) and are presented in table 9 and 10.

3.3.2.2 Variable bioaccumulation factors

Much has been done on the accumulation of heavy metals in earthworms (Ma 1982, Ma et al. 1983, Ma and Van der Voet 1992). It has become clear that soil characteristics such as pH and organic matter can explain a great part of variation in the concentrations in earthworms.
Additional research on metal accumulation in different species of earthworms, influenced by soil characteristics yielded new regression equations (Verhallen and Ma, 1997). Control variables for Cd, Cu and Zn were total heavy metal concentration in the top soil and pH.

$$\log 10([metal]worm) = X_0 + a \cdot \log 10([metal]topsoil) + b \cdot pH$$

where *[metal]worm* = the concentration in earthworms [mg/kg dw]
[metal]topsoil = the total concentration in the top soil [mg/kg dw]

The regression coefficients for the accumulation of metals are presented in table 11 (Verhallen and Ma, 1997)

Table 11: Regression coefficients for accumulation of metals in earthworms

coefficients	X_0	a	b	R^2	n
Cd	2.60	0.49	-0.2	0.69	88
Cu	1.21	0.43	-0.08	0.65	70
Zn	3.07	0.27	-0.1	0.45	87

Table 8: Bioaccumulation factors for Cd for diet items used in the food web model

	geomean	mean	std	n	min	max
Terrestrial:						
leaves	0.27	1.24	1.71	42	0.003	6.03
fruits/buds	0.05	0.09	0.07	7	0.01	0.19
seeds	0.15	0.6	0.77	20	0.001	2.81
roots	0.27	0.33	0.24	8	0.15	0.87
worms	2.70	3.0	1.42	7	1.25	5.13
snails	9.82	15.1	16.4	15	3.42	49.5
spiders	1.76	7.4	13.3	48	0.16	56.7
insect larvae	4.24	6.41	5.89	5	1.03	15
caterpillars	1.23	1.33	0.57	4	0.67	2
insect adult	0.56	2.03	3.59	81	0.04	6.75
birds (as food)	0.053	Data after Jongbloed et al., 1994				
mammals (as food)	0.034	Data after Jongbloed et al., 1994				
Aquatic:						
snail	235	678	828	7	7	8585
crustaceans	338	15115	42808	12	10	150000
insect larvae	12845	15174	9855	6	5400	32857
insect adult	455	455		1	455	
amphibians	3656	No data, as fish				
fish	3656	9132	15743	6	1233	41000

BCF soil to diet item is based on mg/kg dw soil and mg/kg ww diet item, BCF water to diet item is based on mg/l and mg/kg ww diet item, BCF food to diet item is based on mg/kg ww food and mg/kg ww diet item.

Table 9 : Bioaccumulation factors for copper of food items in the food web model

	geomean	mean	std	n	min	max
Terrestrial:						
leaves	0.003	0.004	0.002	7	0.0009	0.006
fruits/buds	0.003	No data, as leaves				
seeds	0.003	No data, as leaves				
roots	0.003	No data, as leaves				
worms	0.15	0.2	0.2	5	0.08	0.19
snails	0.15	No data, as worms				
spiders	0.17	0.33	0.36	27	0.009	1.38
insect larvae	0.15	No data, as worms				
caterpillars	0.08	No data, as insect adult (terr)				
insect adult	0.08	0.14	0.14	26	0.009	0.59
birds (as food)	0.004[1]					
mammals (as food)	0.004[1]					
Aquatic:						
snail	700			1		
crustaceans	15000			1		
insect larvae	3884	4827	3501	4	1440	9651
insect adult	388[2]					
amphibians	1216	No data, as fish				
fish	1216	2104	2644	7	322	7750

BCF soil to diet item is based on mg/kg dw soil and mg/kg ww diet item, BCF water to diet item is based on mg/l and mg/kg ww diet item, BCF food to diet item is based on mg/kg ww food and mg/kg ww diet item.

1 = Calculated with BCF values for liver, kidney, muscle and bone of 0.14, 0.01. 0.006 and 0.006, respectively. The % of body weight are 6, 1.6, 73 and 19.3, for liver, kidney, muscle and bone, respectively. BCF total (DW) is 0.014 and BCF total (WW) is 0.004.

2 = It is assumed that 90% is excreted between larval and adult stage (Timmermans, 1991).

Table 10: Bioaccumulation factors for zinc of food items in the food web model

	geomean	mean	std	n	min	max
Terrestrial:						
leaves	0.004	0.006	0.004	7	0.001	0.12
fruits/buds	0.004	No data, as leaves				
seeds	1.04	1.11	0.38	5	0.53	1.46
roots	0.004	No data, as leaves				
worms	0.76	0.87	0.51	8	0.34	1.85
snails	0.76	No data, as worms				
spiders	0.16	0.24	0.23	27	0.03	1
insect larvae	0.76	No data, as worms				
caterpillars	0.08	No data, as insect adult (terr)				
insect adult	0.08	0.16	0.27	25	0.009	1.28
birds (as food)	0.25[1]					
mammals (as food)	0.25	No data, as for birds				
Aquatic:						
snail	2146	No data, as for fish				
crustaceans	751	992	916	2	344	1640
insect larvae	3809	4139	2075	4	2692	7115
insect adult	2666[2]					
amphibians	1246	No data, as fish				
fish	1246	2478	1356	7	974	4462

BCF soil to diet item is based on mg/kg dw soil and mg/kg ww diet item, BCF water to diet item is based on mg/l and mg/kg ww diet item, BCF food to diet item is based on mg/kg ww food and mg/kg ww diet item.

1 = Calculated with BCF values for liver, kidney, muscle and bone of 1.39, 0.22, 0.22 and 2.98, respectively. The % of body weight are 6, 1.6, 73 and 19.3, for liver, kidney, muscle and bone, respectively. BCF total (DW) is 0.82 and BCF total (WW) is 0.25.

2 = it is assumed that 30% is excreted between larval and adult stage (Timmermans, 1991).

3.3.3 Calculate PEC for target species

The potential environmental concentration (PEC) of birds and mammals, i.e. the exposure concentration, is the average concentration in the food, neglecting exposure by way of drinking water or inhaled air. Exposure of prey items that are into contact with soil is calculated with constant bioaccumulation factors (BAFs) for those prey items where bioavailability equations are not yet available. For earthworms and intermediate prey that feed on earthworms, the BAF is calculated for each grid cell, depending on total heavy metal concentration and pH (*cf.* §3.6).
Water exposure of prey items such as aquatic insects is taken into account by using a single median concentration for all water bodies in The Netherlands (no GIS data available) and fixed bioconcentration factors for such prey items. The aquatic routes are a small minority of the exposure routes and therefore this is considered to be of a minor shortcoming of the method.
PECs were calculated for each grid cell, for those target species that are associated with the nature target type for that specific location:

$$PEC_{TargetSpecies} = \sum_{i=1,n} fDietItem_i \cdot BAF_i \cdot [metal]_{env}$$

where $fDietItem_i$ = diet fraction of item i (to n items)
BAF_i = bioaccumulation factor for diet item i
$[metal]_{env}$ = the concentration of heavy metal in soil or water

3.4 Calculate PEC/NEC ratio for target species

After completing the calculations for the NEC of target species, and the PEC for target species in each grid cell of the National Ecological Network, the PEC/NEC ratio is calculated for each target species at a specific location *j*. A target species *i* is considered to be potentially affected if the PEC/NEC ratio is larger than one, otherwise it is considered to be not affected:

$$\frac{PEC_i}{NEC} > 1 \Rightarrow \text{ potentially affected species } i$$

$$\frac{PEC_i}{NEC} < 1 \Rightarrow \text{ species } i \text{ not affected}$$

3.5 Calculate the Potentially Affected Fraction (PAF) for target species

3.5.1 Potentially affected fraction

The PEC/NEC ratio has been calculated for all avian and mammalian target species in each geographical unit of the national ecological network. The fraction of the species that is potentially affected by a metal, is the number of target species with a PEC/NEC ratio greater than 1, divided by the total number of target species for a specific location *j*:

$$PAF_{TargetSpecies,j} = \frac{nPot.affected_j}{nTargetSpecies_j}$$

3.5.2 Correction for background values

According to Klepper and Van de Meent (1997) the fraction of heavy metals associated with the mineral fraction is considered to be the background level, which will not be taken into account in the risk assessment. Therefore, the PAF is calculated twice, once for the background concentrations ($PAF_{background}$) and once for the total concentrations in the soil (PAF_{total}). In this way the anthropogenic PAF can be distinguished from the background PAF by using the formula:

$$PAF_{anthropogenic} = \left(PAF_{total} - PAF_{background}\right) / \left(1 - PAF_{background}\right)$$

3.5.3 Calculating the combined PAF for Cd, Cu and Zn

The PAF can also be calculated for a group of compounds by either using concentration addition or effect addition (Hamers et al. 1996). This PAF is called the Combi PAF. In this report a Combi PAF for cadmium, zinc and copper will be presented, based on effect addition. This Combi PAF is calculated by:

$$PAF_{combi} = 1 - \left(\left(1 - PAF_{zinc}\right) \cdot \left(1 - PAF_{copper}\right) \cdot \left(1 - PAF_{cadmium}\right)\right)$$

The possible values of a PAF for target species depend on the number of target species per Nature Target Type. For a Nature Target Type with only 10 target species, the PAF can only be 0, or multiples of 0.1 with a maximum value of 1.0. Calculation of the Combi-PAF for several metals can lead to values of PAF that are not exact multiples of 0.1. This is shown in the example below.

Example:
A Nature Target Type consists of 10 avian and mammalian target species. 6 of which are exposed to diet concentrations above their NEC for all three metals, i.e.:
PAF Target Species for cadmium = 0.6
PAF Target Species for zinc = 0.6
PAF Target Species for copper = 0.6

Combi PAF-TS = 1-(0.4*0.4*0.4) = 1-0.064 = 0.936
The nearest possible PAF for target species = 0.9

These combi-PAF value should be considered as estimates for the true number of affected target species without a strict physical reality. For Nature Target Types with a larger number of target species, the difference between the possible values of PAF and the calculated combi-PAF value will be smaller and therefore, the mapping error negligible. This is not the case for small numbers of target species per target type. Due to the use of PAF-classes in mapping the PAF, this will not lead to a significant difference in interpretation of the PAF map.

4. RESULTS

4.1 Calculated No Effect Concentrations for Birds and Mammals

By extrapolating the limited set of toxicity data from laboratory testing, a No Effect Concentration (NEC) for target species can be calculated. Since the extrapolation factors depend on the diet of a particular target species, each species has a different NEC. By plotting the cumulative, we can inspect the resulting sensitivity distribution of target species (Figure 7) . It must be remembered, that this curve is ***not*** the cumulative of the laboratory toxicity data (cf. Klepper and Van de Meent, 1997), but the cumulative of ***calculated*** NECs for target species based on a small set of laboratory data.

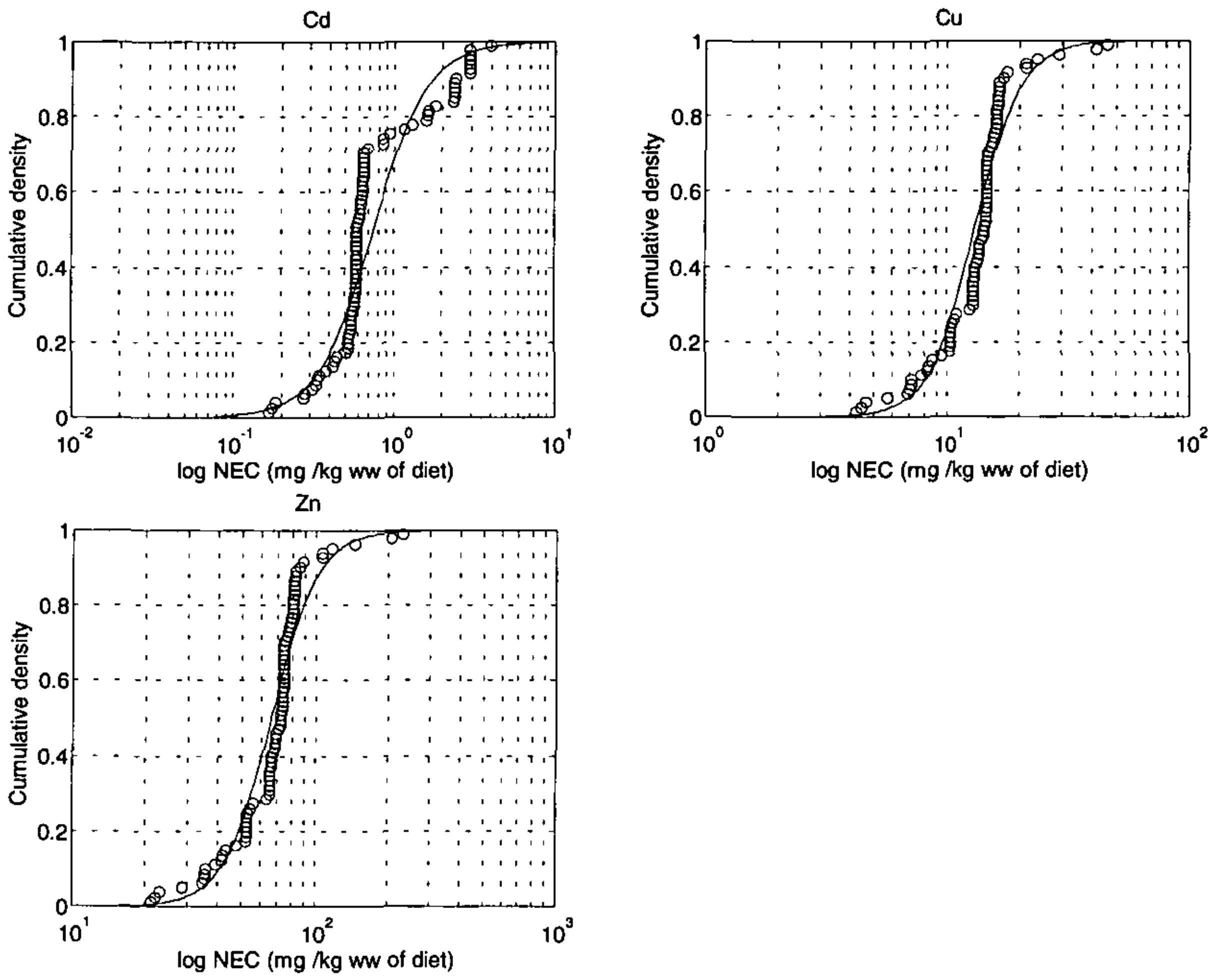


Figure 7: Cumulative No Effect Concentrations (NEC, calculated with the SIGMA model) for birds and mammals for the heavy metals Cadmium, Copper and Zinc. Data: open circles. Drawn line: theoretical cumulative logistic distribution.

The mean calculated cadmium NEC for birds and mammals is 0.85 mg/kg ww of diet (range 0.17 to 6.3 mg/kg diet (ww). The average NEC for copper is ± 12.9 mg/kg diet (ww) and the range is 4.2 to 46 mg/kg diet (ww). The average NEC for zinc is 65 mg/kg diet (ww) and the range is 21 to 230 mg/kg diet (ww). For copper and zinc the most sensitive target species are a factor 10 more sensitive than the least sensitive target species, for cadmium this factor is approximately 40.
The cumulative curve of cadmium is not so smooth as the curves for zinc and copper. This is due to the fact that for cadmium, two different data sets for toxicity were used, one for birds and an other one for mammals. Mammals are less sensitive than birds. Most NECs at the upper right part of the cadmium cumulative are for mammals. For zinc and copper, very few studies were available so a combined data set was used as a starting point for NEC calculations.

4.2 Exceedance of selected PAF levels

For each unit of the National Ecological Network (NEN), a PAF for target species was calculated. With this information, it is possible to calculate the area of the NEN where certain quality objectives, related to PAF are exceeded. In order to make a comparison between the different metals, three levels of PAF were chosen to represent risk limits:

Very low Risk Limit: PAF = 0.0005 units
Low Risk Limit : PAF = 0.05 units
Serious Risk Limit: PAF = 0.5 units

The percentage of the area within the NEN where these three risk limits are exceeded was calculated for the background metal concentrations, the total (present) metal concentrations and the anthropogenic fraction of the metal in soil (Table).
In general, PAFs for target species are already quite high for estimated background concentrations of the three metals, especially for zinc. The total PAF is much higher for cadmium but only marginally so for copper and zinc. The Anthropogenic PAF is highest for cadmium, where even the 'serious risk' limit of 0.5% PAF is reached in 0.8% of the NEN. For Zinc, the 'low risk' limit (anthropogenic PAF > 0.05) is exceeded in 6.7% of the NEN. For copper, only 2.0% of the NEN exceeds the 'low risk' limit of the anthropogenic PAF.

Table 12: Area of the National Ecological Network where specific risk limits are exceeded

CADMIUM	*PAF > 0.0005*	*PAF > 0.05*	*PAF > 0.5*
Background	73.4	62.7	0.0
Total	97.3	93.8	2.1
Anthropogenic	78.4	62.5	0.8

COPPER	*PAF > 0.0005*	*PAF > 0.05*	*PAF > 0.5*
Background	65.5	47.1	0.0
Total	66.7	48.1	0.0
Anthropogenic	3.9	2.0	0.0

ZINC	*PAF > 0.0005*	*PAF > 0.05*	*PAF > 0.5*
Background	98.2	73.5	0.0
Total	98.5	75.7	0.0
Anthropogenic	14.5	6.7	0.0

Nota bene:

Due to extrapolation error, the estimated background PAF can sometimes be higher than the total metal concentration, which is based on extrapolation of measurements. This occurred in 1.5% of the gridcells of the NEN for cadmium, 3.9% for zinc and 0.03% for copper. Grid cells where higher background concentrations were estimated than the extrapolated total metal concentrations, are not taken into account for the calculation of the anthropogenic PAF.

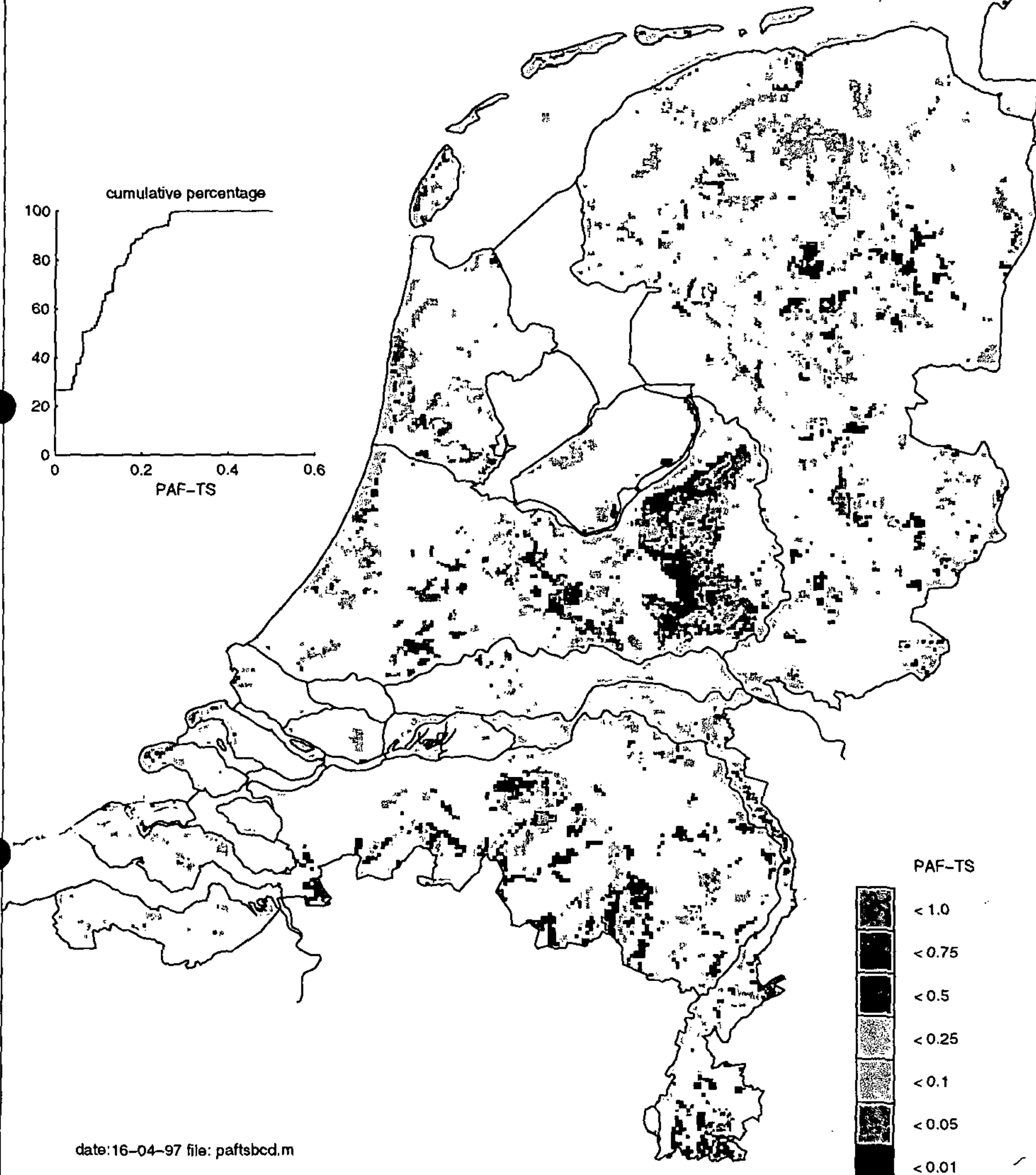
4.3 Mapping the Potentially Affected Fraction of avian and mammalian target species

4.3.1 Cadmium

In figure 8, 9 and 10 the mapping results of cadmium are presented for the background, the total and the anthropogenic concentrations, respectively. The PAFs for the background concentrations are already quite high in the western and northern parts of the Netherlands and are relatively low in the more sandy soils of the Netherlands. Based on total concentration the PAF is greater than 0.1 in most parts of the Netherlands, except in the dunes of Noord-Holland and the isles of the Waddensea, the Waddensea itself and some parts of Drenthe. High anthropogenic PAFs can be found in the sandy soils of the Netherlands but especially in the Kempen.

In figure 11 the same results (a smaller sample) are presented in cumulative fractions of Cd concentrations (figure 11b), of PAF levels (figure 11c) and as a scatter plot of the Cd concentrations versus the PAF. From this figure it can be concluded that the anthropogenic contribution to the PAF (based on total concentration) is substantial.

Background Cd PAF for Target Species



date:16–04–97 file: paftsbcd.m

Figure 8: Background cadmium PAFs for mammalian and avian target species.

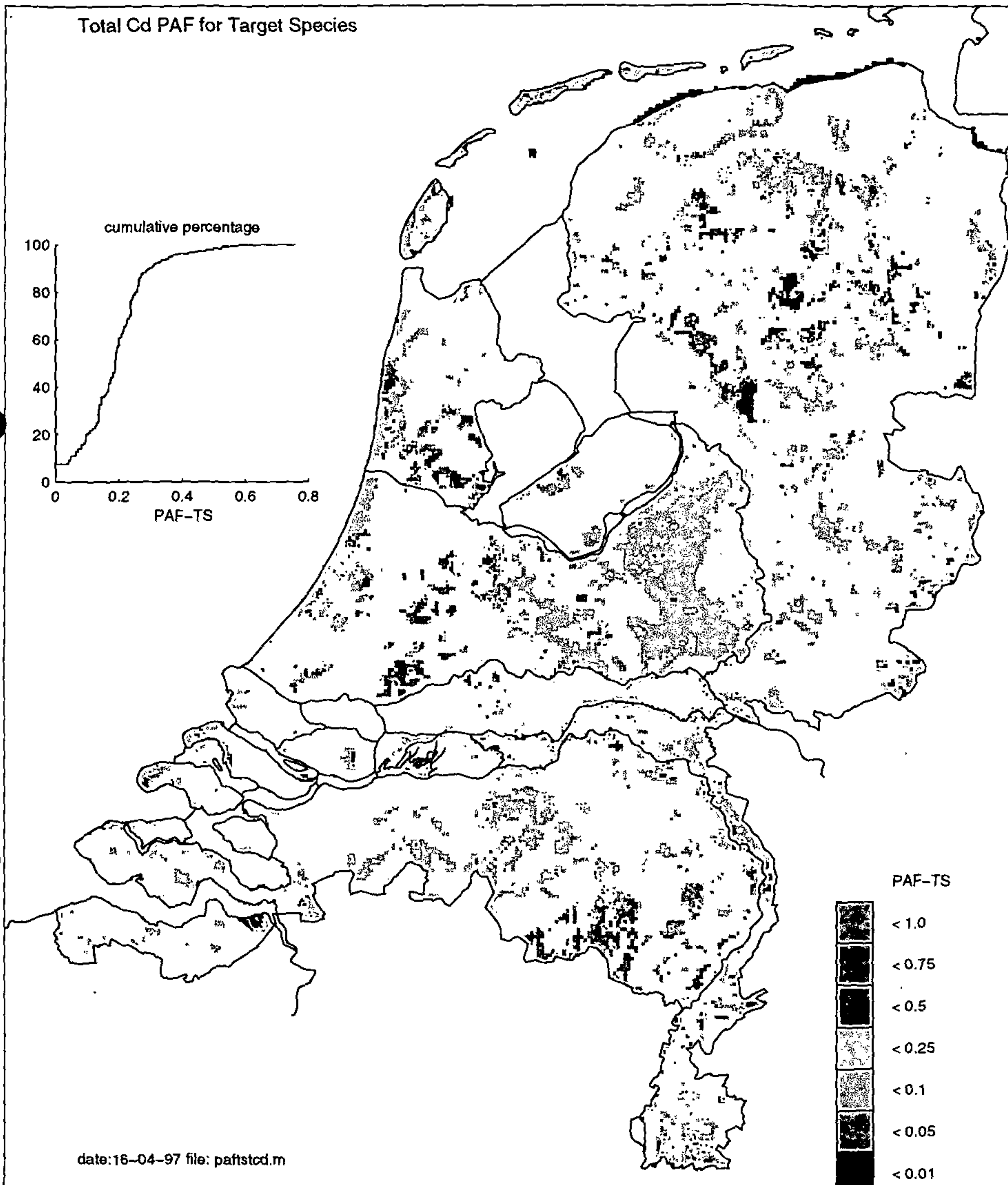
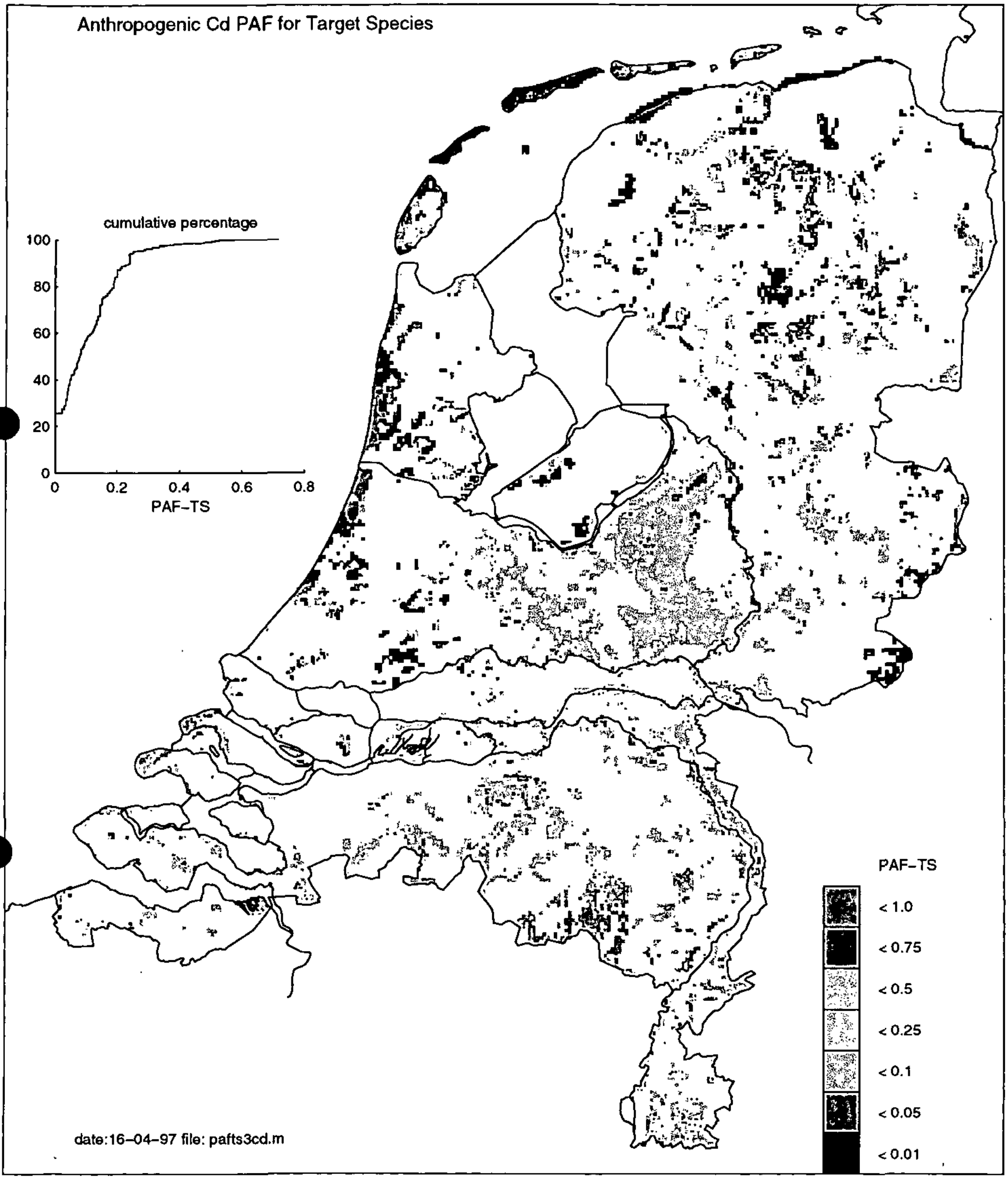

Total Cd PAF for Target Species
cumulative percentage
100
80
60
40
20
0
0
0.2
0.4
0.6
0.8
PAF-TS
PAF-TS
< 1.0
< 0.75
< 0.5
< 0.25
< 0.1
< 0.05
< 0.01
date:16-04-97 file: paftstcd.m

Figure 9: Total cadmium PAFs for mammalian and avian target species.



Anthropogenic Cd PAF for Target Species
cumulative percentage
100
80
60
40
20
0
0
0.2
0.4
0.6
0.8
PAF–TS
PAF–TS
< 1.0
< 0.75
< 0.5
< 0.25
< 0.1
< 0.05
< 0.01
date:16–04–97 file: pafts3cd.m

Figure 10: Anthropogenic cadmium PAFs for mammalian and avian target species.

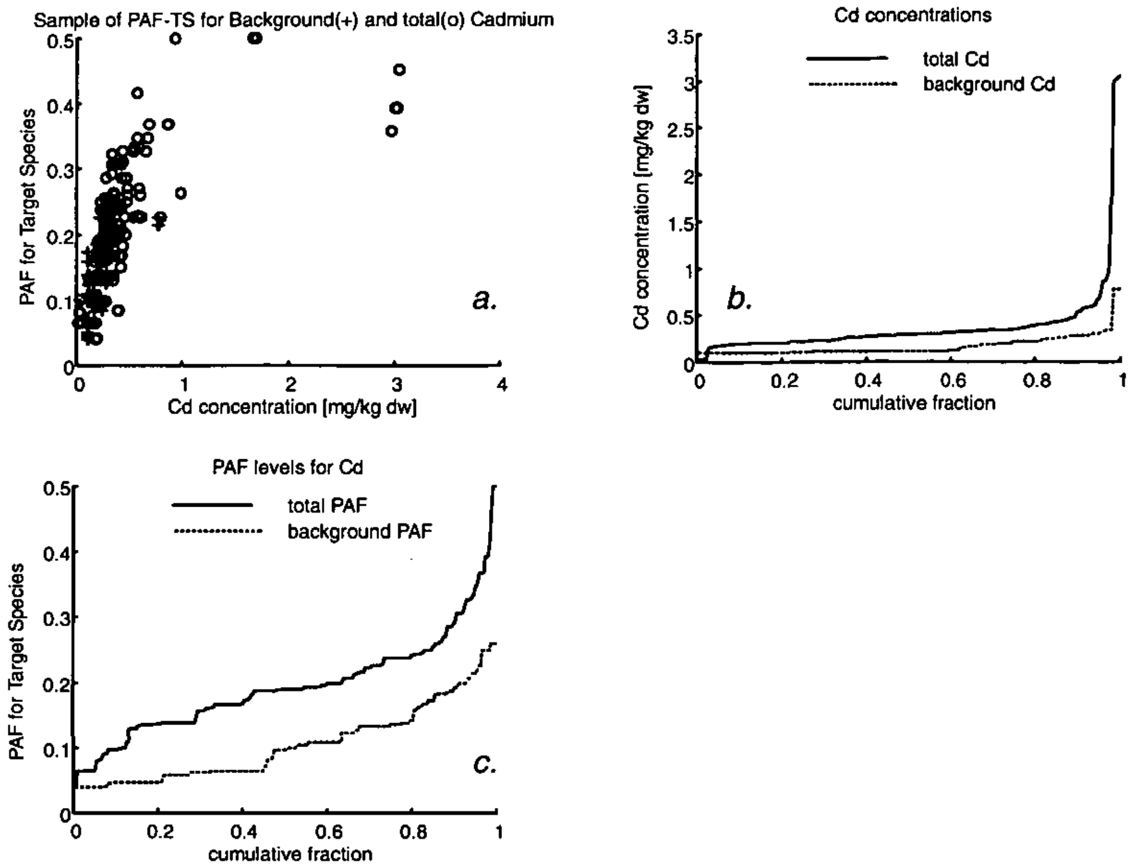


Figure 11: Random sample of total and background Cd concentrations in the NEN ((figure 10.b), associated background and total PAF (Figure 10.c) and a scatter plot of concentration versus PAF (Figure 10.a).

4.3.2 Copper

In figure 12, 13 and 14 the mapping results of copper are presented for the background, the total and the anthropogenic concentrations, respectively. The PAFs for the background concentrations are relative high in the dunes and along the greater rivers. The PAFs for the total concentrations are the same as for the background concentration (same place, same magnitude). Therefore, the the anthropogenic PAFs for copper are really low (<0.01).
In figure 15 the same results (a smaller sample) are presented in cumulative fractions of Cu concentrations (figure 15b), of PAF levels (figure 15c) and as a scatter plot of the Cu concentrations versus the PAF. From this figure it can be concluded that the anthropogenic contribution to the PAF (based on total concentration) is almost nil.

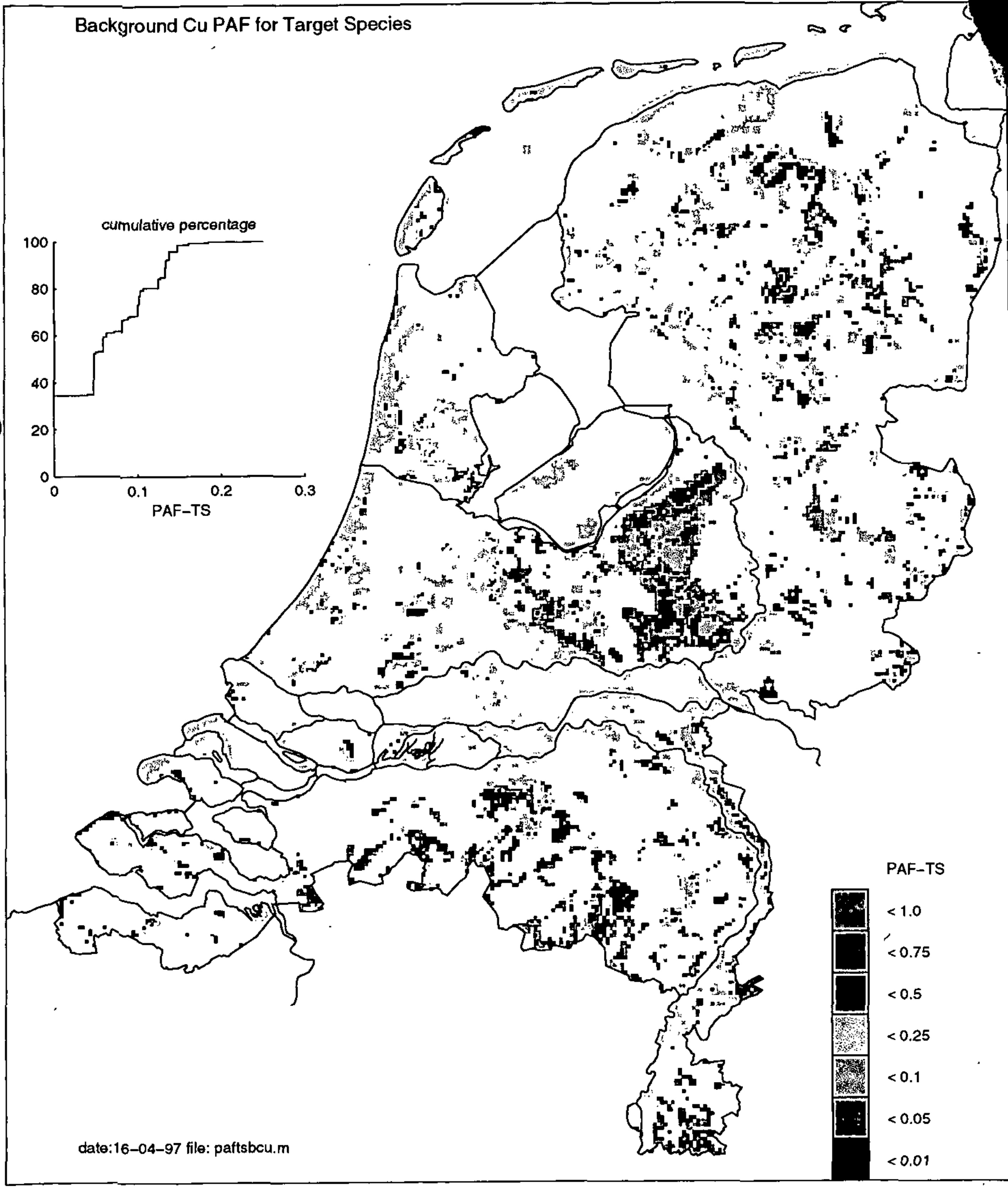

Background Cu PAF for Target Species
cumulative percentage
100
80
60
40
20
0
0
0.1
0.2
0.3
PAF–TS
PAF–TS
< 1.0
< 0.75
< 0.5
< 0.25
< 0.1
< 0.05
< 0.01
date:16-04-97 file: paftsbcu.m

Figure 12: Background copper PAFs for mammalian and avian target species.

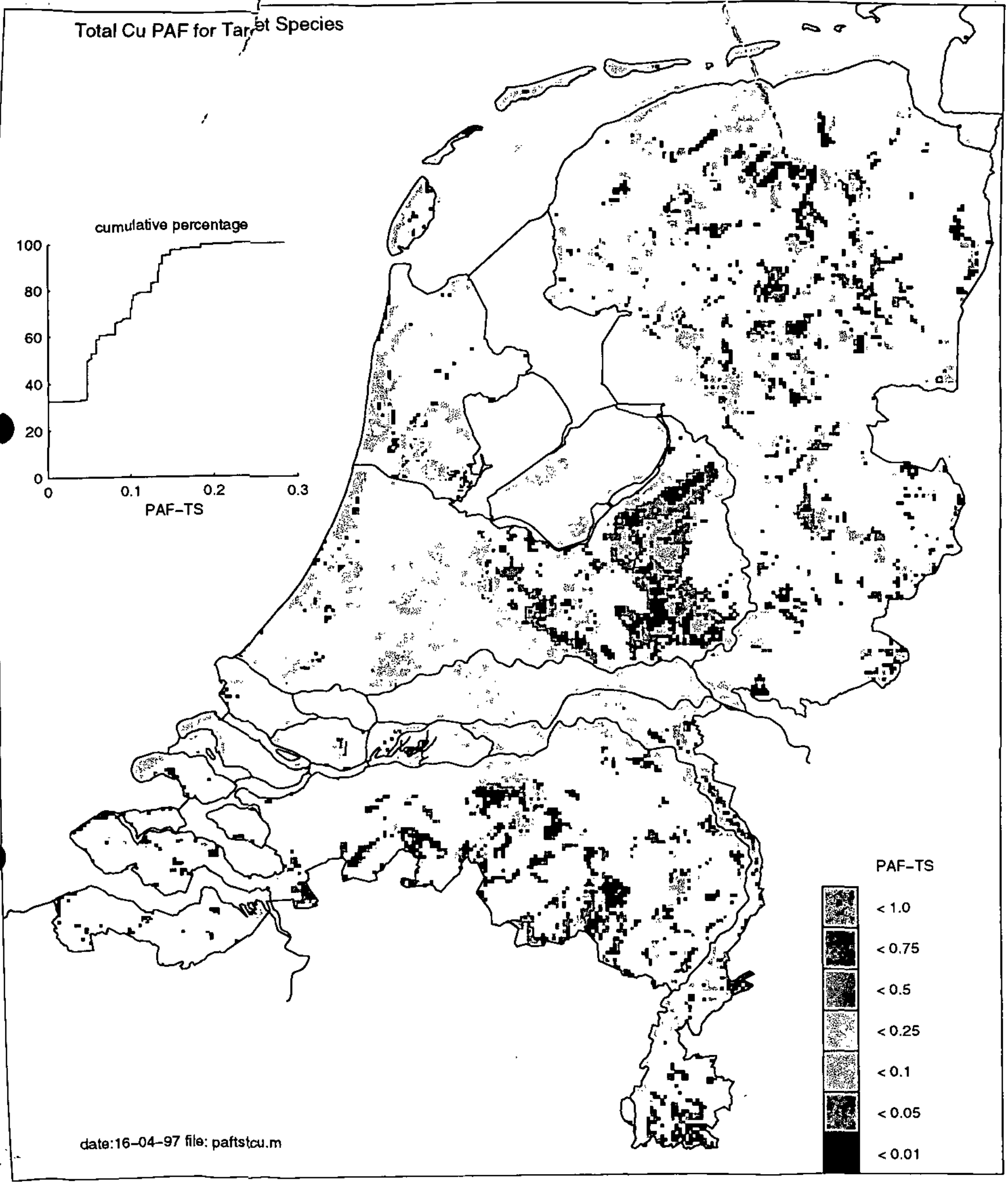


Figure 13: Total copper PAFs for mammalian and avian target species.

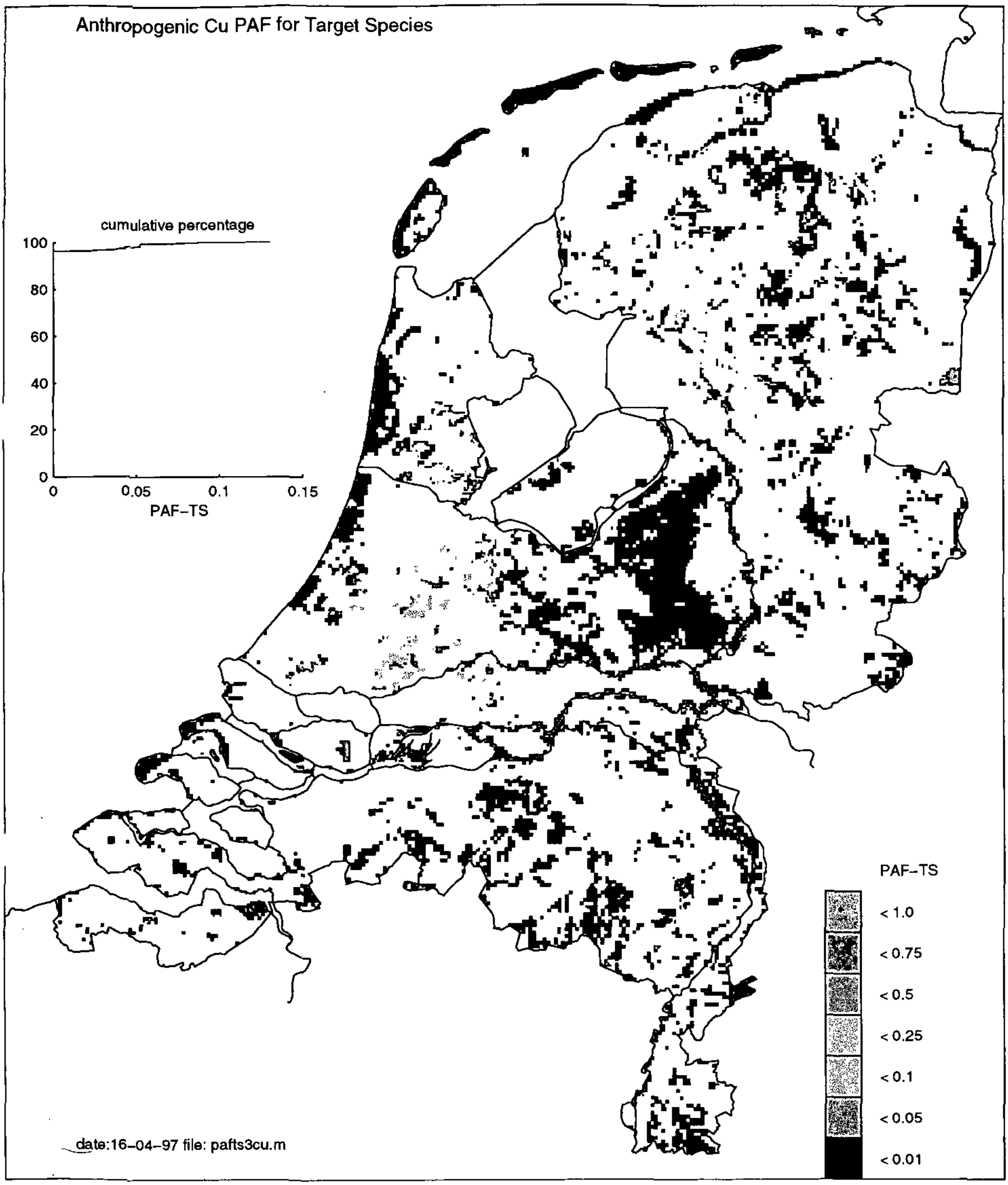


Figure 14: Anthropogenic copper PAFs for mammalian and avian target species.

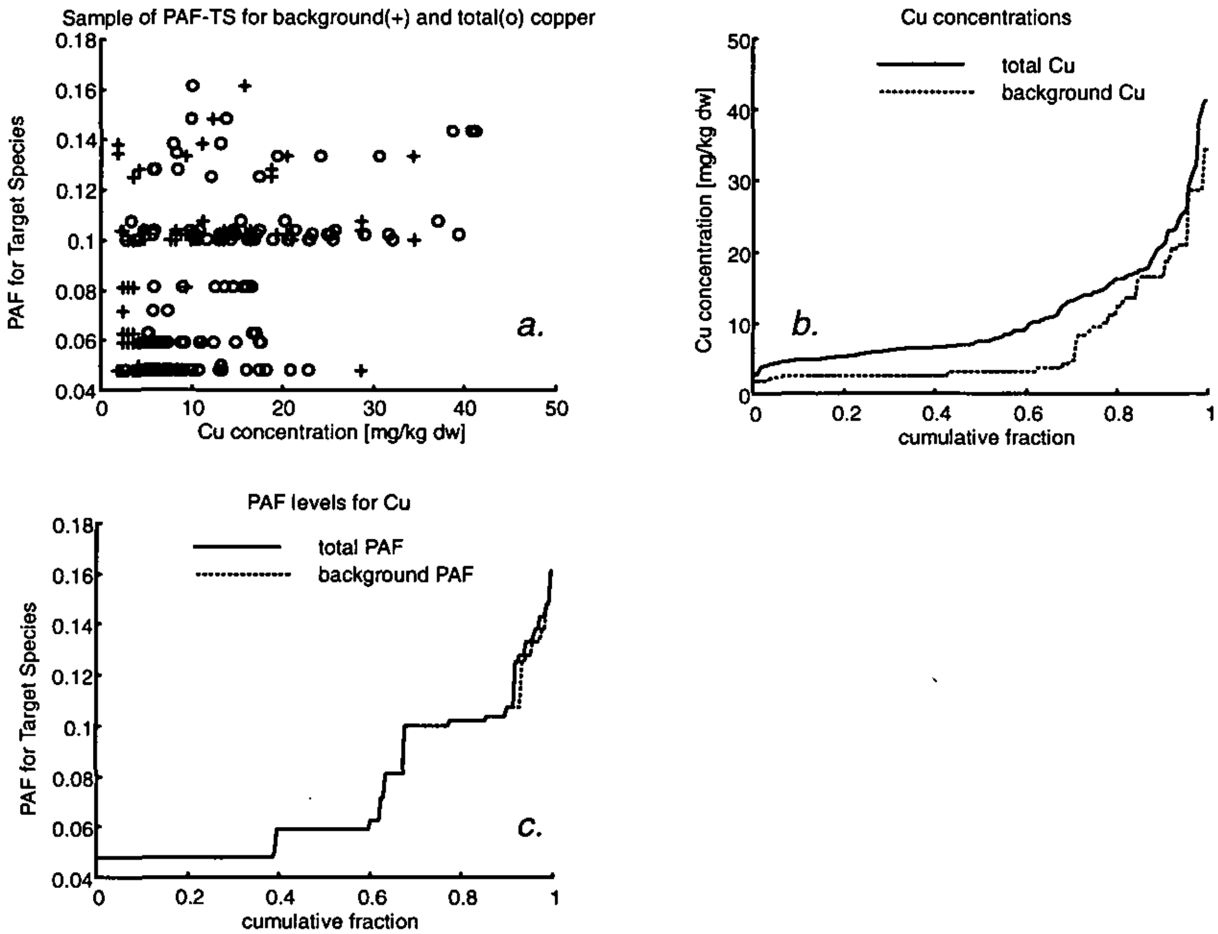


Figure 15: Random sample of total and background Cu concentrations in the NEN (Figure 15.b), associated background and total PAF (Figure 15.c) and a scatter plot of concentration versus PAF (Figure 15.a).

4.3.3 Zinc

In figure 16, 17 and 18 the mapping results of zinc are presented for the background, the total and the anthropogenic concentrations, respectively. The PAFs for the background concentrations are relative high in the peat areas of the western and northern parts of the Netherlands and along the greater rivers. The PAFs for the total concentrations are on the average slightly bit higher in the western peat areas of the Netherlands and the same in the other regions as for the background concentration. Therefore, only elevated PAFs for the anthropogenic contribution are found in the western peat areas.

In figure 19 the same results (a smaller sample) are presented in cumulative fractions of Cu concentrations (figure 19b), of PAF levels (figure 19c) and as a scatter plot of the Cu concentrations versus the PAF. From this figure it can be concluded that the anthropogenic contribution to the PAF (based on total concentration) is very low.

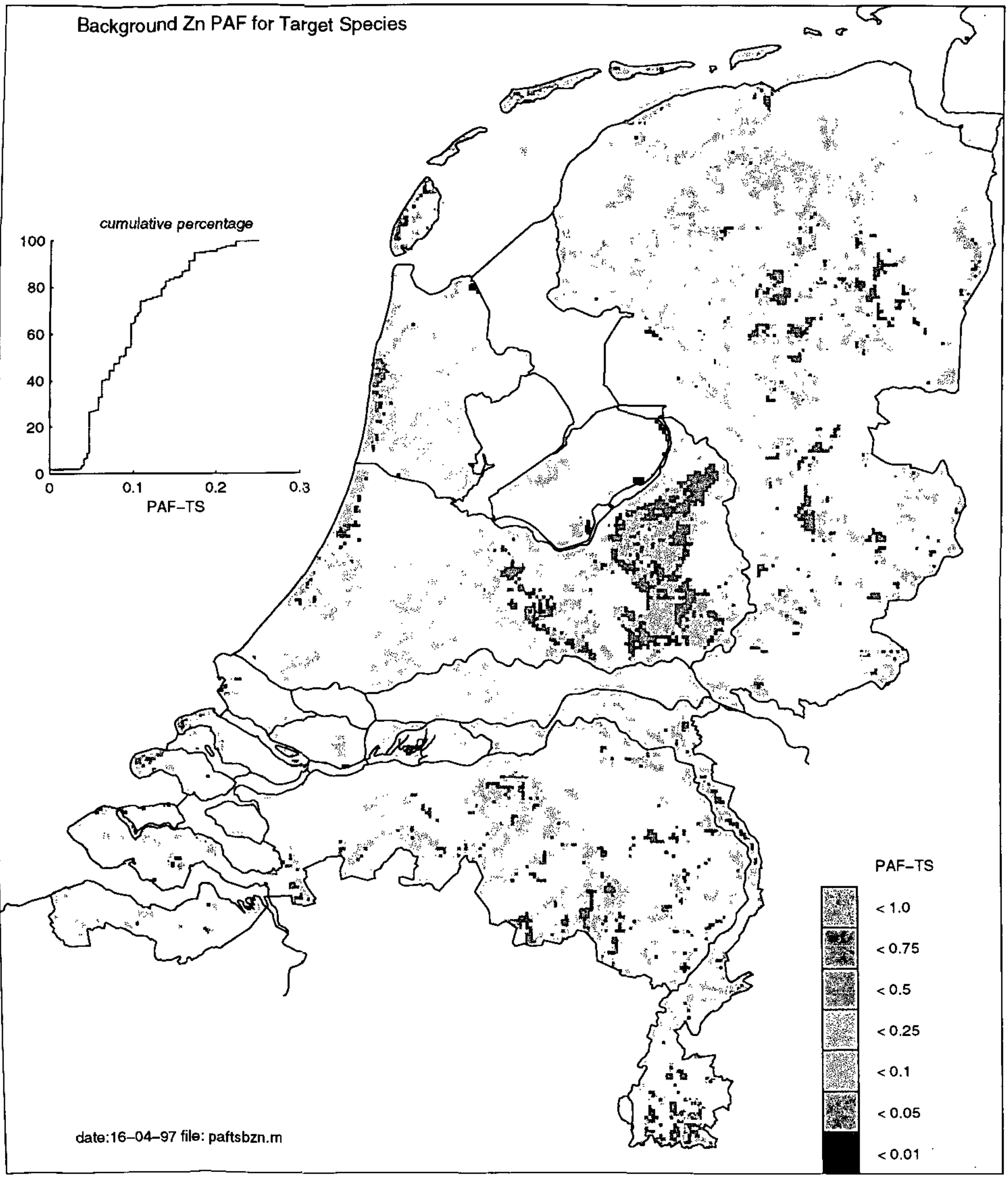


Figure 16: Background zinc PAFs for mammalian and avian target species.

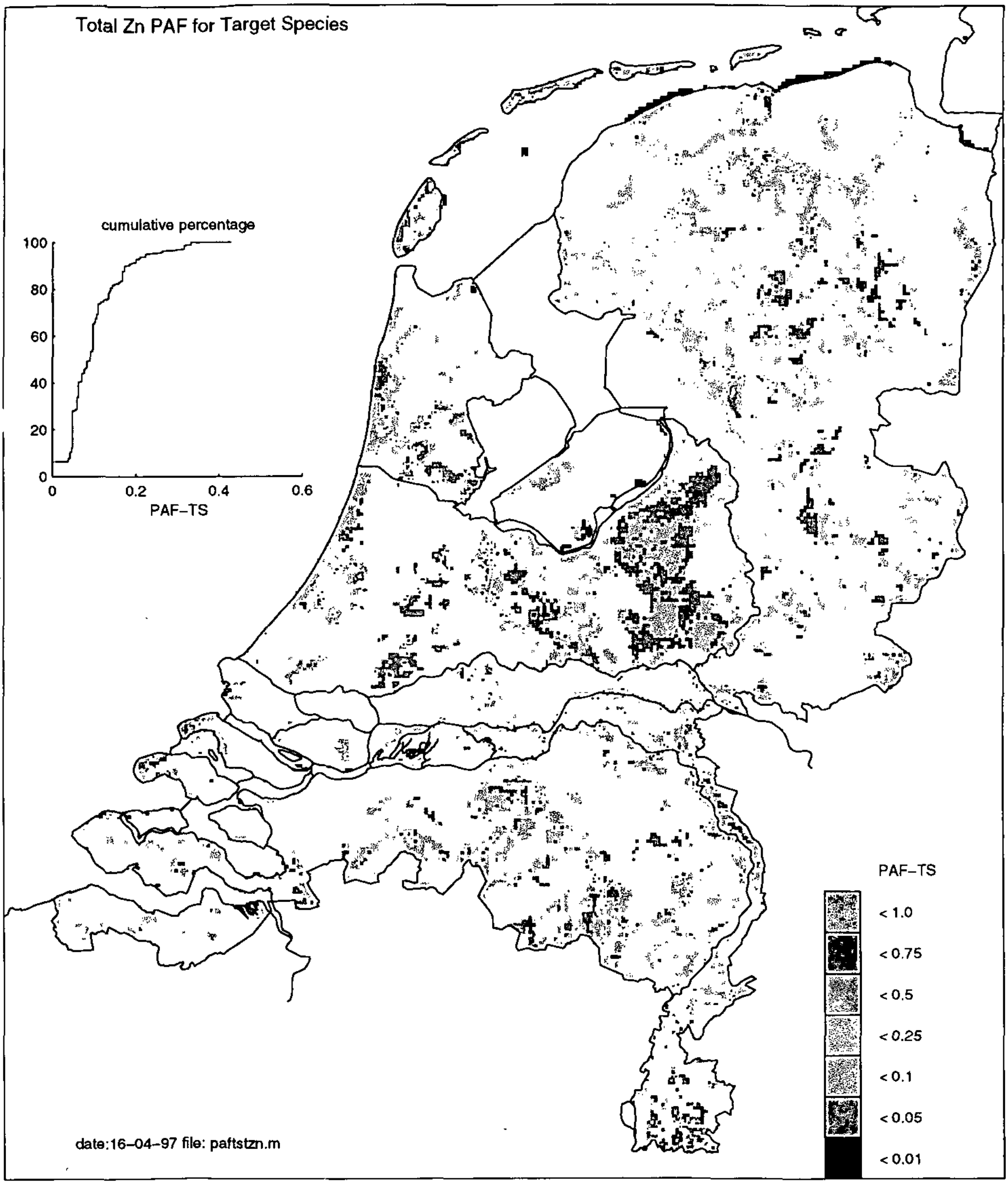


Figure 17: Total zinc PAFs for mammalian and avian target species.

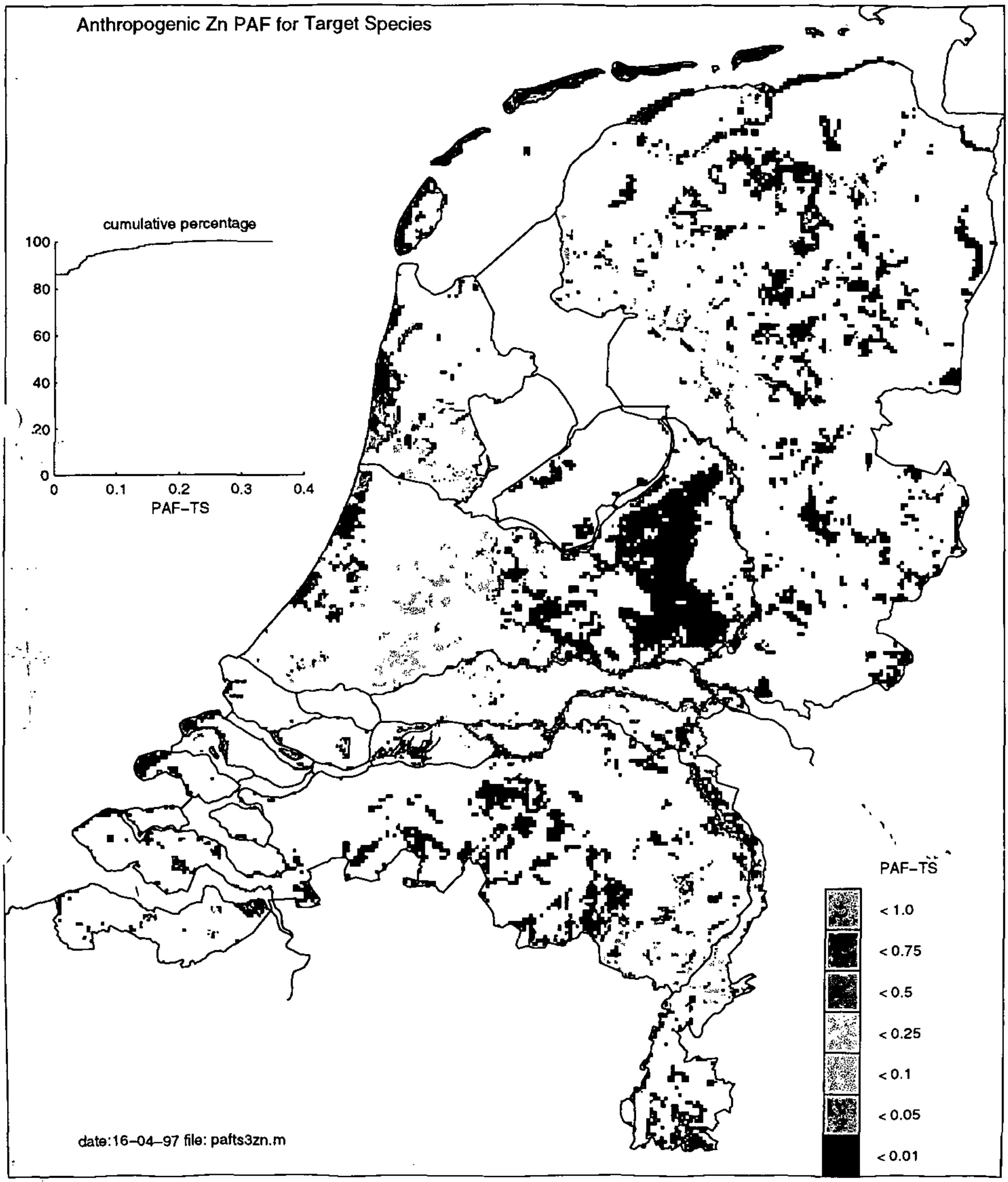


Figure 18: Anthropogenic zinc PAFs for mammalian and avian target species.

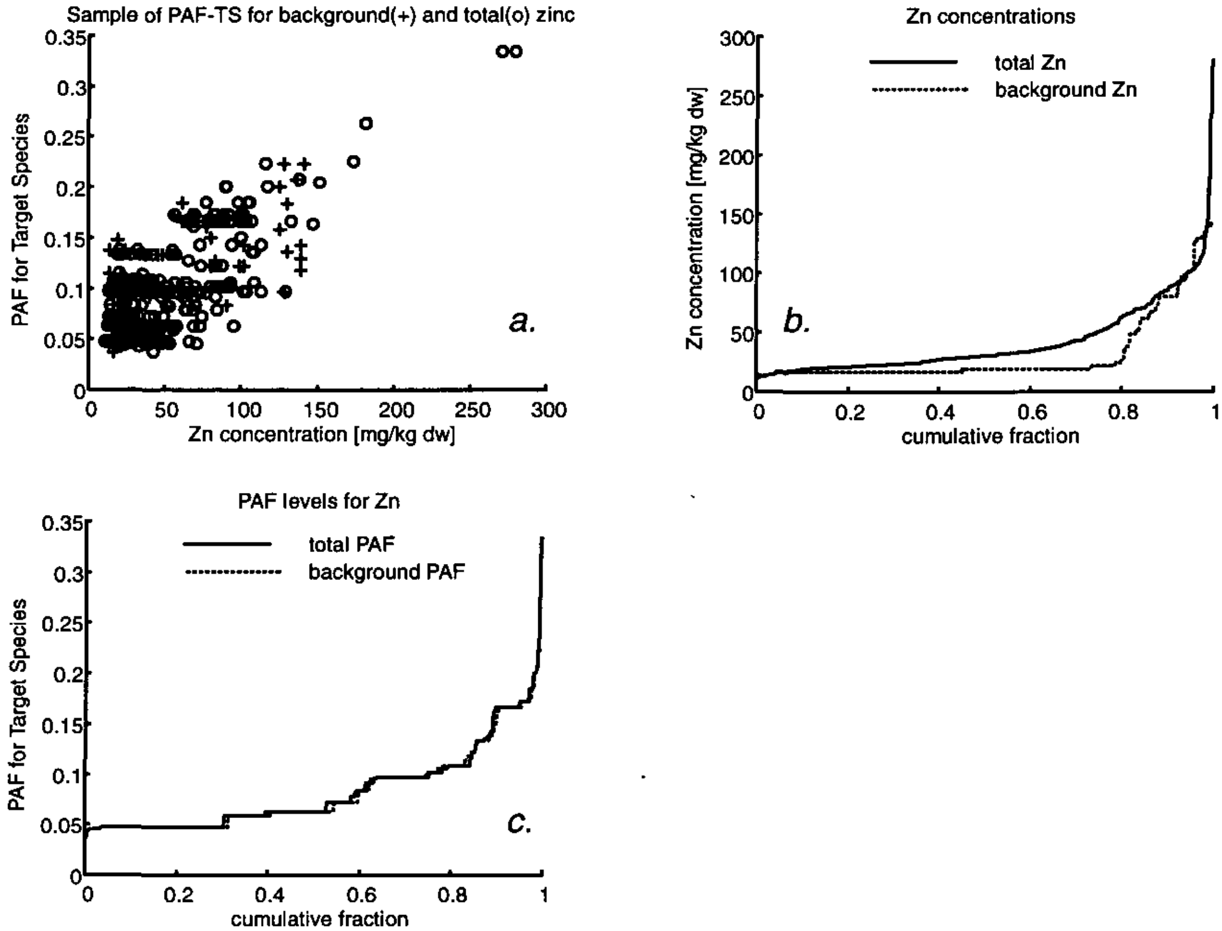


Figure 19: Random sample of total and background Cu concentrations in the NEN (Figure 19.b), associated background and total PAF (Figure 19.c) and a scatter plot of concentration versus PAF (Figure 19.a).

4.3.4 Combi PAF for cadmium, copper and zinc

In figure 20 the anthropogenic combi PAF (based on effect addition) for cadmium, copper and zinc is presented. The outcome of the mapping is to a great extent the same as for cadmium High anthropogenic PAFs are found in the sandy soils of the Netherlands but especially in the Kempen. In addition, elevated PAFs for the anthropogenic contribution are found in the western peat areas (see zinc).

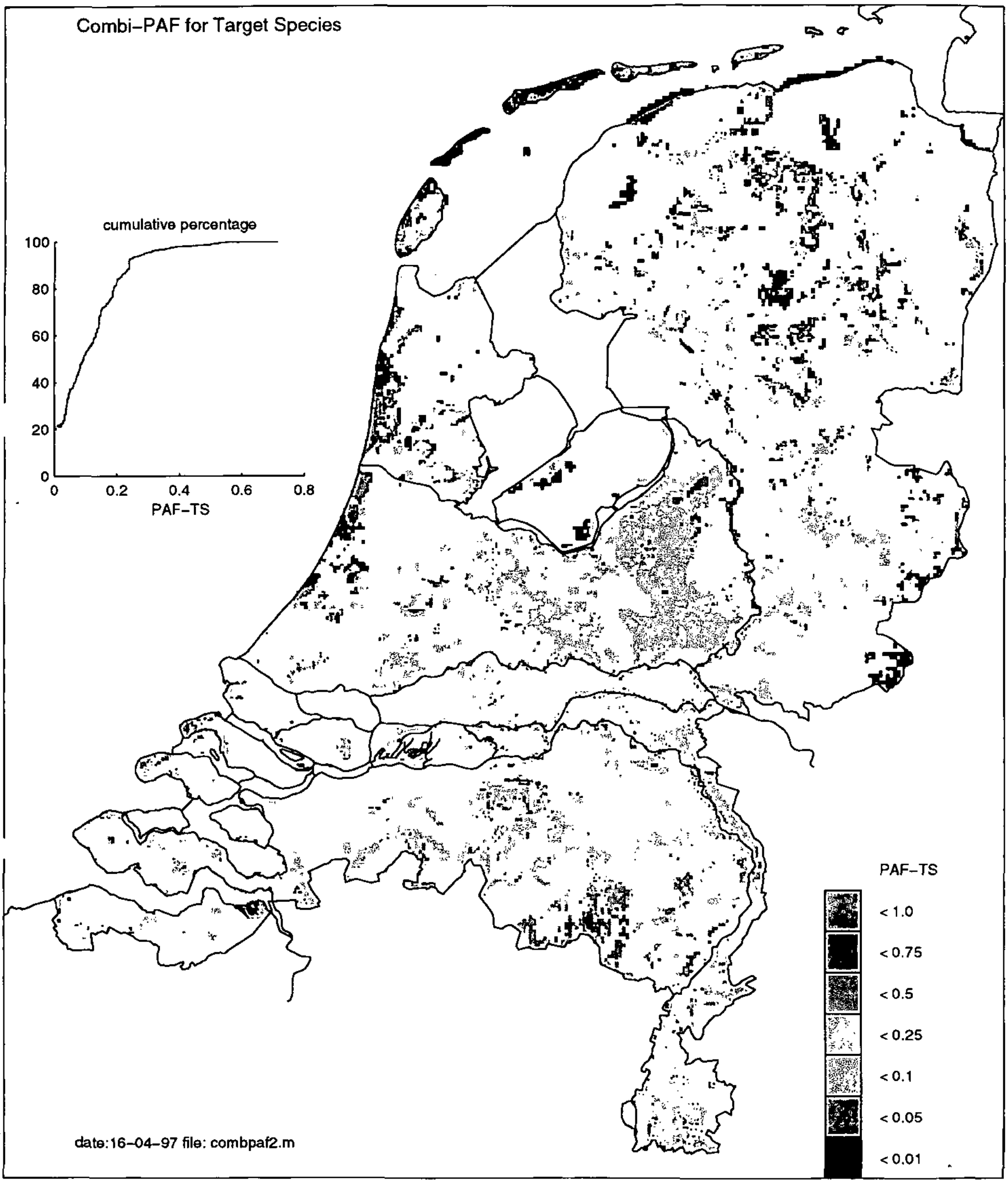


Figure 20: Combi PAFs for the metals cadmium, copper and zinc for mammalian and avian target species.

5. DISCUSSION

When calculated PAF values indicate that a considerable fraction of the species is exposed above the NOEC, the question arises what this means in terms of field effects.
The NOEC is generally an underestimate of the concentrations where effects start to occur (by definition the NOEC should be a tested concentration and, therefore, the underestimation depends on the design of the test, i.e. the gap between the dosages).
An other important factor is that although the endpoints (e.g. growth, reproduction, and mortality) are chosen because they are providing information about populations, the translation to population level-effect is not straightforward. In practice, a decrease in reproduction need not be meaningful if a species population level is determined by food availability (Ferson et al., 1996): the population may only be affected at reproduction rates close to zero. In addition, field populations may be (genetically) adapted: in the laboratory a species shows negative effects, but in the field the organism has time for physiological adaptation, or a resistant population develops by selection.
Against these arguments pointing to a conservative nature of the PAF, there are some reasons to expect field effects at lower concentrations than laboratory NOECs. In the first place, "chronic" tests have in fact a short duration and effect could occur after longer exposure, perhaps even after several generations. Of particular concern in this respect are effects on reproduction which may occur at toxic stress levels well below the NOEC for commonly determined endpoints (Kime et al., 1997). In the second place the median estimate (50th percentile) of the NEC distribution for a particular target species is used. This is certainly not a conservative approach. A worst case approach would have been the use of the 5th percentile In addition, for all the other parameters used in the foodweb, median or mean estimates are used (for example: BCF values and the composition of the diet).
Metals in the environment will be partly or largely of natural origin. The background concentration of zinc and copper, as estimated by Klepper and Van de Meent (1997), leads to noticable background PAFs for target species (Figs. 14 and 18). This may be due to the fact that the concept of species sensitivities distribution (see e.g. Aldenberg & Slob 1992) is used for both essential and non-essential metals. Some assumptions of this method may not hold for essential metals, since too low concentrations of copper or zinc can lead to metal deficiency (Janus et al. 1989, Janus 1993). It also possible that the concept is correct in predicting that some organisms are very sensitive to copper or zinc and that these organisms would be affected at Dutch background levels. However, the procedure devised by Klepper and Van de Meent (1997) rules out their influence by only considering the (estimated) anthropogenic PAF, i.e. the fraction of PAF *in addition* to background PAF. Additional research is needed to clarify the method for essential metals.

6. CONCLUSION

The model applied in this report only provides the number of target species (expressed in percentages) that are exposed at concentration above the NECs of the target species. The model does not give an estimation of the extent of the effects to be expected in the field (for example 45% effect on reproduction). In the model, median estimates were used for BCFs and diet composition. More conservative approaches have been used for the metabolic rate and the toxicity (NOEC concept). The translation of the endpoints to effects on population levels is still not adequate, since no population models were used.
However, at the highest PAFs significant toxic stress seems probable, but validation of these results is needed.

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Appendix

Diet table in (% of weight) for the target species (1-80) and intermediate species (81-94).

Legend:

Number	Species	Abbreviation	Latin or English name
1	Baardmannetje	Bama	Panurus biarmicus
2	Blauwborst	Blbo	Luscinia svecica
3	Blauwe kiekendief	Blki	Circus cyaneus
4	Bontbekplevier	Bopl	Charadrius hiaticula
5	Dodaars	Doda	Tachybaptus ruficollis
6	Draaihals	Drha	Jynx torquilla
7	Duinpieper	Dupi	Anthus campestris
8	Dwergstern	Dwst	sterna albifrons
9	Eidereend	Eide	Somateria mollissima
10	Geelgors	Gego	Emberiza citrinella
11	Geoorde fuut	Gefu	Podiceps nigricollis
12	Grauwe gans	Grga	Anser anser
13	Grauwe gors	Grgo	Miliara calandra
14	Grauwe kiekendief	Grki	Circus pygargus
15	Grauwe klauwier	Grkl	Lanius collurio
16	Griel	Grie	Burhinus oedicnemus
17	Groene specht	Grsp	Picus viridis
18	Grote karekiet	Grka	Acrocephalus arundinaceus
19	Grote stern	Grst	Sterna sandvicensis
20	Grutto	Grut	Limosa limosa
21	Hop	Hop	Upupa epops
22	IJsvogel	Ijsv	Alcedo atthis
23	Kemphaan	Keha	Philomachus pugnax
24	Kerkuil	Keui	Tyto alba
25	Klapekster	Klek	Lanius excubitor
26	Kleine plevier	Klpl	Charadrius dubius
27	Kluut	Klut	Recurvirostra avosetta
28	Korhoen	Koho	Tetrao tetrix
29	Krooneend	Kree	Netta rufina
30	Kuifleeuwerik	Kule	Galerida cristata
31	Kwak	Kwak	Nycticorax nycticorax
32	Kwartelkoning	Kwko	Crex crex
33	Lepelaar	Lepl	Platalea leucorodia
34	Nachtzwaluw	Nazw	Caprimulgus europaeus
35	Noordse stern	Nost	Sterna paradisaea
36	Oeverzwaluw	Oezw	Riparia riparia
37	Ooievaar	Ooiv	Ciconia ciconia
38	Ortolaan	Orto	Emberiza hortulana
39	Paapje	Paap	Saxicola rubetra
40	Patrijs	Patr	Perdix perdix
41	Pijlstaart	Pijl	Anas acuta
42	Porseleinhoen	Poho	Porzana porzana
43	Purperreiger	Pure	Ardea purpurea

44	Raaf	Raaf	Corvus corax
45	Rietzanger	Riza	Acrocephalus schoenobaenus
46	Rode wouw	Rowo	Milvus milvus
47	Roerdomp	Rodo	Botaurus stellaris
48	Roodborsttapuit	Rota	Saxicola torquata
49	Roodkopklauwier	Rokla	Lanius senator
50	Slobeend	Slob	Anas clypeata
51	Snor	Snor	Locustella luscinioides
52	Steenuil	Stui	Athena noctua
53	Strandplevier	Stpl	Charadrius alexandrinus
54	Tapuit	Tapu	Oenanthe oenanthe
55	Torenvalk	Tova	Falco tinnunculus
56	Tureluur	Tulu	Tringa totanus
57	Velduil	Veui	Asio flammeus
58	Visdiefje	Vidi	Sterna hirundo
59	Waterral	Wara	Rallus aquaticus
60	Watersnip	Wasn	Gallinago gallinago
61	Wielewaal	Wiwa	Oriolus oriolus
62	Woudaapje	Woaa	Ixobrychus minutus
63	Zomertaling	Zota	Anas querquedula
64	Zwarte stern	Zwst	Chlidonias niger
65	Boommarter	Boma	Martes martes
66	Bruinvis	Brvi	Phocoena phocoena
67	Das	Das	Meles meles
68	Eikelmuis	Eimu	Eliomys quercinus
69	Franjestaart	Frst	Myotis natteri
70	Gewone zeehond	Geze	Phoca vitulina
71	Grote hoefijzerneus	Grho	Rhinolophus ferrumequinum
72	Hamster	Hams	Cricetus cricetus
73	Ingekorven vleermuis	Invl	Myotis emarginatus
74	Kleine hoefijzerneus	Klho	Rhinolophus hipposideros
75	Mopsvleermuis	Movl	Barbastella barbastellus
76	Noordse woelmuis	Nowo	Microtus oeconomus
77	Otter	Otte	Lutra lutra
78	Tuimelaar	Tuim	Tursiops truncatus
79	Vale vleermuis	Vavl	Myotis myotis
80	Waterspitsmuis	Wasp	Neomys fodiens
81	Vogel/klein	Vokl	Bird/small
82	Vogel/middel	Vomi	Bird/medium size
83	Vogel/groot	Vogr	Bird/large
84	Reptiel/klein	Rekl	Reptile/small
85	Reptiel/groot	Regr	Reptile/large
86	Amfibie/klein	Amkl	Amphibian/small
87	Amfibie/groot	Amgr	Amphibian/large
88	Vis/klein	Vikl	Fish/small
89	Vis/middel	Vimi	Fish/medium size
90	Vis/groot	Vigr	Fish/large
91	Zoogdier/zeer klein	Zozk	Mammal/very small
92	Zoogdier/klein	Zokl	mammal/small
93	Zoogdier/vrij klein	Zovk	Mammal/medium size
94	Zoogdier/groot	Zogr	Mammal/large

Species abbreviation	Bama	Blbo	Blki	Bopl	Doda	Drha	Dupi	Dwst	Eide	Gego
Species number	1	2	3	4	5	6	7	8	9	10
Leaves	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
fruits/buds	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
seeds	6.6	0.7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	84.8
roots	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
worms	0.0	0.0	0.0	49.9	0.0	0.0	0.0	1.5	0.0	0.0
snails(terr)	68.7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
spiders	10.3	2.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
insect larvae (terr)	10.3	2.2	0.0	0.0	0.0	71.4	0.0	0.0	0.0	0.0
caterpillars	0.0	94.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
insect adult (terr)	4.1	0.9	0.0	0.3	0.0	28.6	100.0	0.0	0.0	15.2
birds small	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
birds medium size	0.0	0.0	9.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0
birds large	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
mammals very small	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
mammals small	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
mammals medium size	0.0	0.0	37.9	0.0	0.0	0.0	0.0	0.0	0.0	0.0
mammals large	0.0	0.0	52.6	0.0	0.0	0.0	0.0	0.0	0.0	0.0
snail (aq)	0.0	0.0	0.0	35.6	6.6	0.0	0.0	0.0	98.1	0.0
crustaceans	0.0	0.0	0.0	14.2	0.0	0.0	0.0	23.5	1.9	0.0
insect larvae (aq)	0.0	0.0	0.0	0.0	0.8	0.0	0.0	0.0	0.0	0.0
insect adult (aq)	0.0	0.0	0.0	0.0	39.5	0.0	0.0	0.0	0.0	0.0
amphibians	0.0	0.0	0.0	0.0	26.3	0.0	0.0	0.0	0.0	0.0
fish	0.0	0.0	0.0	0.0	26.8	0.0	0.0	75.0	0.0	0.0

Species abbreviation	Gefu	Grga	Grgo	Grki	Grkl	Grie	Grsp	Grka	Grst	Grut
Species number	11	12	13	14	15	16	17	18	19	20
Leaves	0.3	20.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
fruits/buds	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
seeds	0.0	48.0	98.1	0.0	0.0	0.0	0.0	0.0	0.0	0.1
roots	0.0	32.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
worms	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	43.4
snails(terr)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	8.8	0.0	8.7
spiders	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.3	0.0	0.0
insect larvae (terr)	0.0	0.0	0.0	0.0	0.0	39.7	21.7	22.6	0.0	21.7
caterpillars	0.0	0.0	0.0	0.0	0.0	12.7	0.0	0.0	0.0	0.0
insect adult (terr)	0.0	0.0	1.9	1.4	9.7	47.6	78.3	26.9	0.0	26.1
birds small	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
birds medium size	0.0	0.0	0.0	9.0	0.0	0.0	0.0	0.0	0.0	0.0
birds large	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
mammals very small	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
mammals small	0.0	0.0	0.0	0.0	90.3	0.0	0.0	0.0	0.0	0.0
mammals medium size	0.0	0.0	0.0	89.6	0.0	0.0	0.0	0.0	0.0	0.0
mammals large	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
snail (aq)	13.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
crustaceans	10.6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
insect larvae (aq)	0.9	0.0	0.0	0.0	0.0	0.0	0.0	0.5	0.0	0.0
insect adult (aq)	47.8	0.0	0.0	0.0	0.0	0.0	0.0	39.8	0.0	0.0
amphibians	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
fish	27.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	100.0	0.0

Species abbreviation	Hop	IJsv	Keha	Keui	Klek	Klpl	Klut	Koho	Kree	Kule
Species number	21	22	23	24	25	26	27	28	29	30
Leaves	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.5	1.5	0.3
fruits/buds	0.0	0.0	0.0	0.0	0.0	0.0	0.0	99.0	97.6	69.1
seeds	0.0	0.0	2.1	0.0	0.0	0.0	0.0	0.5	0.0	4.5
roots	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.0	0.0
worms	0.0	0.0	0.0	0.0	0.0	0.0	23.0	0.0	0.0	3.5
snails(terr)	0.0	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	0.0	0.0	10.5	0.0	0.0	0.0	0.0
insect larvae (terr)	62.0	0.0	5.6	0.0	0.0	14.0	0.0	0.0	0.0	0.0
caterpillars	13.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	22.1
insect adult (terr)	24.8	0.0	1.8	0.0	1.9	5.6	0.0	0.1	0.0	0.6
birds small	0.0	0.0	0.0	0.0	7.9	0.0	0.0	0.0	0.0	0.0
birds medium size	0.0	0.0	0.0	7.0	0.0	0.0	0.0	0.0	0.0	0.0
birds large	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
mammals very small	0.0	0.0	0.0	24.0	0.0	0.0	0.0	0.0	0.0	0.0
mammals small	0.0	0.0	0.0	9.0	19.0	0.0	0.0	0.0	0.0	0.0
mammals medium size	0.0	0.0	0.0	54.0	71.2	0.0	0.0	0.0	0.0	0.0
mammals large	0.0	0.0	0.0	6.0	0.0	0.0	0.0	0.0	0.0	0.0
snail (aq)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
crustaceans	0.0	2.4	0.0	0.0	0.0	0.0	46.0	0.0	0.0	0.0
insect larvae (aq)	0.0	0.2	1.1	0.0	0.0	0.0	0.3	0.0	0.0	0.0
insect adult (aq)	0.0	12.0	89.4	0.0	0.0	69.9	30.7	0.0	0.0	0.0
amphibians	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
fish	0.0	85.4	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

Species abbreviation	Kwak	Kwko	Lepl	Nazw	Nost	Oezw	Ooiv	Orto	Paap	Patr
Species number	31	32	33	34	35	36	37	38	39	40
Leaves	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	24.6
fruits/buds	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	96.8	0.0
seeds	0.0	0.9	0.0	0.0	0.0	0.0	0.0	21.6	0.5	68.5
roots	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
worms	0.0	14.5	0.0	0.0	0.0	0.0	1.5	0.0	0.0	0.0
snails(terr)	0.0	2.9	0.0	0.0	0.0	0.0	0.3	0.0	0.0	0.0
spiders	0.0	0.4	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
insect larvae (terr)	0.3	29.0	0.0	0.0	0.0	0.0	0.8	0.6	1.9	4.9
caterpillars	0.0	0.0	0.0	0.0	0.0	0.0	0.0	76.8	0.0	0.0
insect adult (terr)	0.6	52.1	0.0	100.0	2.1	100.0	0.9	1.0	0.8	2.0
birds small	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
birds medium size	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
birds large	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
mammals very small	0.0	0.0	0.0	0.0	0.0	0.0	18.4	0.0	0.0	0.0
mammals small	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
mammals medium size	0.0	0.0	0.0	0.0	0.0	0.0	45.9	0.0	0.0	0.0
mammals large	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
snail (aq)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
crustaceans	0.2	0.0	5.0	0.0	1.4	0.0	0.0	0.0	0.0	0.0
insect larvae (aq)	0.0	0.0	0.4	0.0	0.0	0.0	0.0	0.0	0.0	0.0
insect adult (aq)	0.5	0.0	18.6	0.0	0.0	0.0	0.0	0.0	0.0	0.0
amphibians	65.0	0.0	0.0	0.0	0.0	0.0	32.2	0.0	0.0	0.0
fish	33.3	0.0	76.0	0.0	96.5	0.0	0.0	0.0	0.0	0.0

Species abbreviation	Pijl	Poho	Pure	Raaf	Riza	Rowo	Rodo	Rota	Rokla	Slob
Species number	41	42	43	44	45	46	47	48	49	50
leaves	0.0	0.7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.2
fruits/buds	0.0	0.0	0.0	1.2	0.0	0.0	0.0	0.0	0.0	0.0
seeds	27.6	12.6	0.0	0.1	0.0	0.0	0.0	0.0	0.0	1.2
roots	3.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
worms	0.0	0.0	0.0	0.0	0.0	0.0	0.0	6.9	0.0	0.0
snails(terr)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
spiders	0.0	2.0	0.0	0.0	0.0	0.0	0.0	2.1	0.0	0.0
insect larvae (terr)	0.0	0.0	0.0	0.1	62.5	0.0	0.0	2.1	0.0	0.0
caterpillars	0.0	0.0	0.0	0.0	0.0	0.0	0.0	88.2	0.0	0.0
insect adult (terr)	0.0	0.0	0.0	0.1	37.5	0.1	0.2	0.8	100.0	0.0
birds small	0.0	0.0	0.0	2.4	0.0	0.9	0.0	0.0	0.0	0.0
birds medium size	0.0	0.0	0.0	0.0	0.0	2.7	0.0	0.0	0.0	0.0
birds large	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
mammals very small	0.0	0.0	0.0	0.0	0.0	2.2	0.0	0.0	0.0	0.0
mammals small	0.0	0.0	0.0	0.0	0.0	4.4	0.0	0.0	0.0	0.0
mammals medium size	0.0	0.0	10.3	36.0	0.0	5.5	0.0	0.0	0.0	0.0
mammals large	0.0	0.0	0.0	60.0	0.0	45.7	0.0	0.0	0.0	0.0
snail (aq)	38.3	65.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	60.9
crustaceans	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	24.4
insect larvae (aq)	0.3	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1
insect adult (aq)	30.7	19.5	0.3	0.0	0.0	0.0	0.3	0.0	0.0	12.2
amphibians	0.0	0.0	3.6	0.0	0.0	3.7	12.9	0.0	0.0	0.0
fish	0.0	0.0	85.9	0.0	0.0	34.8	86.5	0.0	0.0	0.0

Species abbreviation	Snor	Stui	Stpl	Tapu	Tova	Tulu	Veui	Vidi	Wara	Wasn
Species number	51	52	53	54	55	56	57	58	59	60
Leaves	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.4	0.0
fruits/buds	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
seeds	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.4	0.2
roots	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
worms	0.0	1.3	58.5	0.0	0.0	61.8	0.0	0.7	0.0	99.3
snails(terr)	12.9	0.0	0.0	0.0	0.0	0.0	0.0	0.0	15.6	0.0
spiders	1.9	0.0	0.0	38.5	0.0	0.0	0.0	0.0	0.0	0.0
insect larvae (terr)	1.9	0.0	0.3	25.6	0.0	0.8	0.0	0.0	26.0	0.4
caterpillars	82.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
insect adult (terr)	0.8	4.2	0.2	35.9	30.0	0.3	0.0	0.8	31.2	0.2
birds small	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
birds medium size	0.0	0.0	0.0	0.0	4.0	0.0	10.0	0.0	0.0	0.0
birds large	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
mammals very small	0.0	0.0	0.0	0.0	3.0	0.0	0.0	0.0	0.0	0.0
mammals small	0.0	0.0	0.0	0.0	17.0	0.0	10.0	0.0	0.0	0.0
mammals medium size	0.0	94.6	0.0	0.0	46.0	0.0	70.0	0.0	0.0	0.0
mammals large	0.0	0.0	0.0	0.0	0.0	0.0	10.0	0.0	0.0	0.0
snail (aq)	0.0	0.0	14.6	0.0	0.0	30.9	0.0	0.0	0.0	0.0
crustaceans	0.0	0.0	17.6	0.0	0.0	6.2	0.0	0.3	0.0	0.0
insect larvae (aq)	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.5	0.0
insect adult (aq)	0.0	0.0	8.8	0.0	0.0	0.0	0.0	0.0	26.0	0.0
amphibians	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
fish	0.0	0.0	0.0	0.0	0.0	0.0	0.0	98.2	0.0	0.0

Species abbreviation	Wiwa	Woaa	Zota	Zwst	Boma	Brvi	Das	Eimu	Frst	Geze
Species number	61	62	63	64	65	66	67	68	69	70
Leaves	0.0	0.0	0.3	0.0	0.0	0.0	28.0	0.0	0.0	0.0
fruits/buds	26.3	0.0	54.2	0.0	50.0	0.0	7.0	20.1	0.0	0.0
seeds	0.0	0.0	0.3	0.0	0.0	0.0	0.0	1.2	0.0	0.0
roots	0.0	0.0	0.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0
worms	0.0	0.0	0.0	0.0	0.0	0.0	27.0	0.0	0.0	0.0
snails(terr)	0.0	0.0	0.0	0.0	0.0	0.0	1.0	10.0	0.0	0.0
spiders	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	3.6	0.0
insect larvae (terr)	0.0	0.2	0.0	0.0	0.0	0.0	9.0	0.0	0.0	0.0
caterpillars	42.1	0.0	0.0	0.0	0.0	0.0	0.0	6.4	0.0	0.0
insect adult (terr)	31.7	0.4	0.0	0.0	0.0	0.0	9.0	12.0	96.4	0.0
birds small	0.0	0.0	0.0	0.0	10.0	0.0	0.0	50.2	0.0	0.0
birds medium size	0.0	0.0	0.0	0.0	10.0	0.0	2.0	0.0	0.0	0.0
birds large	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
mammals very small	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
mammals small	0.0	0.0	0.0	0.0	15.0	0.0	3.0	0.0	0.0	0.0
mammals medium size	0.0	0.0	0.0	0.0	15.0	0.0	3.0	0.0	0.0	0.0
mammals large	0.0	0.0	0.0	0.0	0.0	0.0	6.0	0.0	0.0	0.0
snail (aq)	0.0	0.0	20.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0
crustaceans	0.0	0.0	16.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0
insect larvae (aq)	0.0	0.0	0.1	0.8	0.0	0.0	0.0	0.0	0.0	0.0
insect adult (aq)	0.0	0.4	8.1	42.0	0.0	0.0	0.0	0.0	0.0	0.0
amphibians	0.0	22.0	0.0	0.0	0.0	0.0	5.0	0.0	0.0	0.0
fish	0.0	77.0	0.0	57.1	0.0	100.0	0.0	0.0	0.0	100.0

Species abbreviation	Grho	Hams	Invl	Klho	Movl	Nowo	Otte	Tuim	Vavl	Wasp
Species number	71	72	73	74	75	76	77	78	79	80
Leaves	0.0	0.5	0.0	0.0	0.0	20.0	0.0	0.0	0.0	0.0
fruits/buds	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
seeds	0.0	2.8	0.0	0.0	0.0	48.0	0.0	0.0	0.0	0.0
roots	0.0	0.9	0.0	0.0	0.0	32.0	0.0	0.0	0.0	0.0
worms	0.0	23.7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
snails(terr)	0.0	4.7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	70.1
spiders	0.0	0.0	83.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0
insect larvae (terr)	0.0	23.7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
caterpillars	0.0	15.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
insect adult (terr)	100.0	28.4	16.7	100.0	100.0	0.0	0.0	0.0	100.0	0.4
birds small	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
birds medium size	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
birds large	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
mammals very small	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
mammals small	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
mammals medium size	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
mammals large	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
snail (aq)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.6	0.0	0.0
crustaceans	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	28.1
insect larvae (aq)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.4
insect adult (aq)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
amphibians	0.0	0.0	0.0	0.0	0.0	0.0	11.4	0.0	0.0	0.0
fish	0.0	0.0	0.0	0.0	0.0	0.0	88.6	99.4	0.0	0.0

Species abbreviation	Vokl	Vomi	Vogr	Rekl	Regr	Amkl	Amgr	Vikl	Vimi	Vigr
Species number	81	82	83	84	85	86	87	88	89	90
Leaves	0.0	0.0	0.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0
fruits/buds	15.0	40.0	93.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
seeds	5.0	10.0	5.6	0.0	0.0	0.0	0.0	0.0	0.0	0.0
roots	0.0	0.0	0.9	0.0	0.0	0.0	0.0	0.0	0.0	0.0
worms	0.0	0.0	0.0	26.4	5.6	26.4	10.2	10.0	0.0	0.0
snails(terr)	0.0	0.0	0.0	52.8	1.1	52.8	2.0	0.0	0.0	0.0
spiders	0.0	0.0	0.0	7.9	0.2	7.9	0.3	0.0	0.0	0.0
insect larvae (terr)	15.0	15.0	0.0	9.2	16.8	9.2	30.5	0.0	0.0	0.0
caterpillars	50.0	15.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
insect adult (terr)	15.0	20.0	0.0	3.7	20.3	3.7	36.6	0.0	0.0	0.0
birds small	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
birds medium size	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
birds large	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
mammals very small	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
mammals small	0.0	0.0	0.0	0.0	56.0	0.0	0.0	0.0	0.0	0.0
mammals medium size	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
mammals large	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
snail (aq)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
crustaceans	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	20.0
insect larvae (aq)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	90.0	100.0	30.0
insect adult (aq)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
amphibians	0.0	0.0	0.0	0.0	0.0	0.0	20.3	0.0	0.0	0.0
fish	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	50.0

Species abbreviation	Zozk	Zokl	Zovk	Zogr
Species number	91	92	93	94
Leaves	0.0	7.0	20.0	0.0
fruits/buds	0.0	0.0	0.0	9.7
seeds	0.0	70.0	48.0	0.6
roots	0.0	0.0	32.0	0.0
worms	52.0	0.0	0.0	0.0
snails(terr)	0.0	0.0	0.0	0.0
spiders	17.0	0.0	0.0	0.0
insect larvae (terr)	13.0	23.0	0.0	0.0
caterpillars	9.0	0.0	0.0	0.0
insect adult (terr)	9.0	0.0	0.0	0.0
birds small	0.0	0.0	0.0	24.2
birds medium size	0.0	0.0	0.0	0.0
birds large	0.0	0.0	0.0	0.0
mammals very small	0.0	0.0	0.0	58.1
mammals small	0.0	0.0	0.0	0.0
mammals medium size	0.0	0.0	0.0	0.0
mammals large	0.0	0.0	0.0	0.0
snail (aq)	0.0	0.0	0.0	0.0
crustaceans	0.0	0.0	0.0	0.0
insect larvae (aq)	0.0	0.0	0.0	0.0
insect adult (aq)	0.0	0.0	0.0	0.0
amphibians	0.0	0.0	0.0	4.8
fish	0.0	0.0	0.0	2.5