Aquatic Ecology **33:** 363–376, 1999.

Transfer of microcontaminants from sediment to chironomids, and the risk for the Pond bat *Myotis dasycneme* (Chiroptera) preying on them

J.O. Reinhold[1], A.J. Hendriks[2,*], L.K. Slager[3] and M. Ohm[4]
[1]*Bat Research Office, P.O. Box 190, 6700 AD Wageningen, The Netherlands;* [2]*Institute for Inland Water Management and Waste Water Treatment, P.O. Box 17, 8200 AA Lelystad, The Netherlands;* [3]*During this study working for: Institute for Environmental Studies, De Boelelaan 1115, 1081 HV Amsterdam, The Netherlands;* [4]*Ministry of Transport, Public Works and Water Management, P.O. Box 556 3000 AN Rotterdam, The Netherlands; *Author for correspondence*

Accepted 25 November 1999

Key words: accumulation, bats, Chironomidae, Chiroptera, food chain transfer, heavy metals, *Myotis dasycneme*, PAHs, PCBs, pesticides, sediment

Abstract

Transfer ratios of metals, PCBs, pesticides and PAHs from the sediment to chironomid larvae and adults collected in a highly contaminated area, the Biesbosch, were studied. Metal concentrations in larvae were 0.28 (Cd), 0.02 (Cr), 0.52 (Cu), 0.06 (Hg), 0.03 (Pb), 0.32 (Zn) times those found in standard sediment, on a dry weight basis. Hg and Zn were well transported to the adult stage. Dry weight ratios of contaminant residues in adults and in larvae were 0.38 (Cd), 0.23 (Cr), 0.62 (Cu), 1.03 (Hg), 0.08 (Pb), 0.94 (Zn). For PCBs and pesticides, the concentration ratios of chironomid larvae fat to sediment (dry organic matter) varied around 3.3, which is consistent with laboratory studies. Organochlorine residues in adult fat were comparable to those in larvae lipids. However, the concentrations of non-ortho PCBs were 1.7 times higher in adults. For polycyclic aromatic hydrocarbons (PAH), larval fat:sediment (organic matter) concentration ratios ranged from 0.004 to 0.1. Adult: larvae ratios for PAHs varied between 0.2 and 0.6. For naphthalene a much higher transport ratio of 2 was found. Chironomid adults are the most important potential food source of the Pond bat, which lives in low densities in the Biesbosch. The contaminant concentrations measured in the chironomids do not exceed diet levels that are thought to be safe for mammals. However, Pond bats collected in less contaminated areas contained PCB-concentrations of 9, 33 and 76 mg kg^{-1} lipid weight, which are above concentrations that cause reproduction effects on Mink.

Introduction

The Dutch Rhine-Meuse delta has been polluted with microcontaminants for many years, and as a result, has become one of the most polluted areas of the Netherlands. One of the most serious problems occurring in this area is the accumulation of contaminants and their subsequent incorporation into food chains. Persistent substances, in particular heavy metals and organochlorine compounds, are known to threaten benthic and piscivore predators such as the Tufted duck *Aythya fuligula*, the Cormorant *Phalacrocorax carbo*, the Badger *Meles meles* and the Otter *Lutra lutra* (Marquenie et al., 1986; Van den Berg et al., 1992; Dirksen et al., 1991; Broekhuizen & De Ruiter-Dijkman, 1988; Ma & Broekhuizen, 1989).

So far, the effects that these contaminants have on bats (Chiroptera) foraging in sedimentation areas of the Rhine-Meuse delta have not been investigated. In this area, however,the population density of one particular species – the Pond bat *Myotis dasycneme* – is low compared to similar, but less polluted areas (Limpens et al., 1997), suggesting that contaminants have a negative effect on its population density. The Pond bat, an internationally endangered bat species, hunts almost exclusively a few decimeters above the water surface of lakes and rivers from April to October. Probably, the Pond bat mainly eats flying Chironomids, a diet

similar to that of the Daubenton's bat (Swift & Racey, 1983), which has comparable foraging behaviour and foraging areas (Limpens et al., 1997). The only difference between the foraging areas of both species is the size of the water surface. The Pond bat prefers large water surfaces; the Daubenton's bat smaller water surfaces. The distance between the hunting area and roost can be large. For the Pond bat of the Biesbosch a distance of more then 20 km was found (Reinhold, 1994). From October to April, the Pond bat hibernates and survives on its fat reserves.

Chironomid larvae living in polluted areas accumulate substantial amounts of contaminants from the sediment (Larsson, 1984; Van Hattum et al., 1993; Timmermans et al., 1989). These pollutants are transported from the larval to the adult phase as described by Timmermans & Walker (1989), Derr & Zabik (1972), Fairchild et al. (1992) and Larsson (1984). By transport via this described food chain, contaminated sediment could therefore form a threat to the Pond bat and insectivore birds.

Our study has been conducted in one particular area within the Rhine-Meuse delta, which includes the Biesbosch, the Hollandsch Diep and the Haringvliet (Figure 1). The aims of this study were to determine:

(1) the degree of transport of contaminants from polluted sediment, via Chironomid larvae to the adult Chironomids;
(2) whether the Pond bat is affected by the concentrations of contaminants present in the adult chironomids that they feed on.

To meet the first objective, sediment samples collected from the Biesbosch were analysed by Mol (1993). Then, an analysis was made of the organochlorine and heavy metal concentrations present in larval and adult chironomids collected from the same locations. To verify the relialibity of these results, they were compared to available data obtained from the Hollandsch Diep.

The degree of transport is expressed in two ratios; firstly, the proportion of contaminants present in the larval Chironomids to those in the sediment, and secondly, the proportion of contaminants present in the adult Chironomids to those in the larvae. As described in Hendriks (1995), each of these two ratios is supposed to be fixed, and not related to the degree of pollution in the area.

Analysis of Biesbosch bats was not possible because bats are protected by Dutch law and no dead bats were found in the area during our study. In order to discuss the possible risks for the Biesbosch bats, the contamination levels measured in the Biesbosch chironomids were compared to results obtained from laboratory experiments on bats that were fed contaminated food, reported in literature. To further complete the discussion as far as possible and in order to include data obtained from bats foraging in polluted fresh water areas, dead Pond bats from other areas have been collected and analysed.

Study area and methods

Study area

The Biesbosch is a fresh water tidal river with a total area of 4300 ha (Figure 1), characterised by little islands dominated by the Nettle *Urtica dioica* and willows *Salix* spec. and surrounded by water from the large Amer and Nieuwe Merwede rivers. On three locations in the Biesbosch, sediment samples were taken and larval and adult chironomids were collected. The locations were scattered over the area and were all situated within the potential hunting area of the Pond bat.

Sampling

In 1992, the upper 15 cm of the sediment was sampled at the three locations, using a box-core (Mol, 1993). At each location, six subsamples of 15 ml each, were taken.

In April 1993, sediment was sucked up at each location and lead through a stainless steel sieve with a mesh-width of 0.5 mm. The remaining chironomids were collected from this sieve and put into containers filled with water collected at each of the locations. To empty the intestinal tract of the larvae, a defecation period of two days was inserted. During this period, each larvae sample was kept in filtered water obtained from its own location. The three samples (about 5 g fresh weight each and containing at least 150 animals) were frozen until chemical analysis.

In May and June of 1993, adult chironomids were caught on the same three locations. During dusk, the layer between the water surface and one meter above the water surface was sampled using a net measuring 40 cm across. This layer is characteristic for the hunting area of the Pond bat. After freezing the three samples, the chironomids were separated from the other insect groups. Each chironomid sample had a fresh weight of around 5 g.

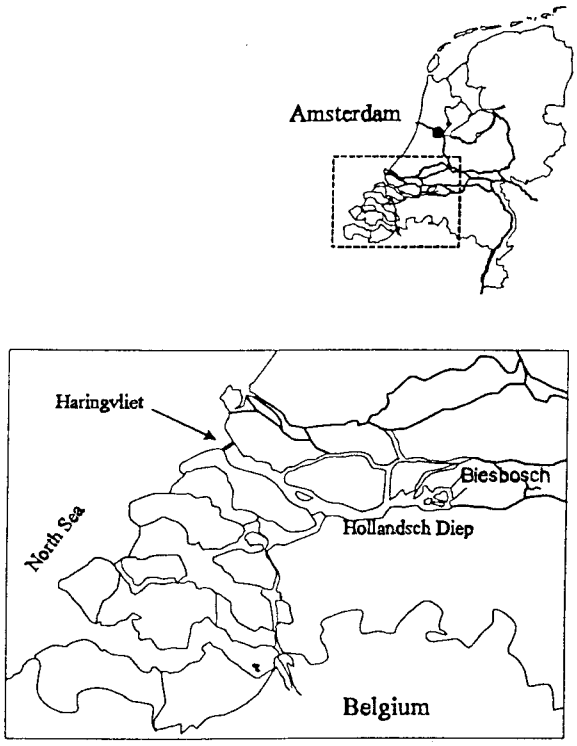

Figure 1. Situation of the Biesbosch in the Netherlands.

A justified and reliable study on the transfer of contaminants from the larval to the adult stage could only be made if the same composition of chironomid species was present in both the larvae and adult samples. Prior to analysing the contaminants, a impression was made of the composition of each of the samples.

Chemical analysis of the sediment

Heavy metals, standard PCBs (IUPAC: 28, 52, 101, 118, 138, 153, 180), organochlorine pesticides and polycyclic aromatic hydrocarbons (PAH) present in the sediment were analysed (Mol, 1993).

Hg analysis was performed by cold vapour atomic absorption spectrometry. Cd, Cr, Cu, Zn, Pb and Ni levels were determined using AAS. To determine the presence and concentration of PCBs and pesticides, three subsamples of 15 ml sediment from each location were homogenised. This homogenate was mixed with acetone and hexane successively. The hexane phase was dried with water-deactivated natriumsulphate and evaporated to 10 ml in a Kuderna Danish evaporator. This extract was further evaporated to 1 ml under a stream of nitrogen gas.

The 1 ml extract was placed onto a column containing 11% water-deactivated basic aluminium oxide (2 g). The purified extract was diluted in 12 ml hexane and evaporated to 0.5–1 ml with nitrogen gas. This 1 ml extract was added to a 5% water-deactivated silica column (1.5 g). The polar pesticides were separated from the PCBs by diluting the extract in 25 ml hexane and 25 ml hexane: diethyl ether (75:25, v/v) successively. Both fractions were evaporated to 1 ml under a stream of nitrogen gas. Each fraction was analysed using GC-electron-capture detection (GC-ECD).

For the PAHs analysis, three samples of 15 ml sediment each were homogenised. 10 g of this sediment sample was shaken with acetone and hexane successively. The hexane phase was dried using water-deactivated natriumsulphate. Samples were analysed using HPLC with fluorescense- and absorption detection.

Comparing the concentrations of heavy metals and organic compounds found in the sediment samples to data of chironomids, the former were corrected to a standard sediment with 25% silt (=fraction $< 16\ \mu$m) and 10% organic matter. To obtain these standards, the heavy metal concentrations were multiplied by 25 and divided by the silt content. Organic compounds were

multiplied by 10 and divided by the organic matter content of the sample (Ministry of transport and public Works, 1989).

Chemical analysis of the chironomids

Chironomids were analysed for heavy metals, PCBs (including non- and mono-ortho), organochlorine pesticides and PAHs.

For the heavy metal analysis, samples of chironomid larvae and adults were freeze-dried and homogenised. Subsamples of 40-100 mg were dissolved in 5 ml HNO_3 (Baker Ultrex, Deventer, The Netherlands) in PTFE acid digestion bombs (PARR-1547). The final distillate was diluted in double-deionized water to a volume of 20 ml. Cd, Cr, Cu, Ni and Pb levels were measured by graphite furnace-atomic absorption spectrometry using deuterium or Zeeman background correction (Perkin Elmer Z3030/HGA 600/AS 60). To prevent interfering matrix effects, a graphite L'vov platform oven and a matrixmodifier were used (Cd and Pb: 2% ammoniumsulphate, 0.1% $Mg(NO_3)_2$; Ni and Cr: 0.5% $Mg(NO_3)_2$, Baker). Zn was analysed by flame atomic absorption spectrometry, using deuterium background correction (Perkin Elmer 4000). Hg was analysed by cold vapour atomic absorption spectrometry with an amalgam system. Electrodeless discharge lamps served as the light source for the Cd and Hg measurements and hollow cathode lamps for the analysis of the other metals.

Chironomid subsamples for PAHs, PCBs and pesticides analysis were extracted by soxhlet extraction. The extract was purified over an alumina column and fractionated on a silica column. PAH analysis was performed using HPCL with fluorescense and absorption detection. Chlorobiphenyls and other organochlorine compounds were determined by gas chromatography using an ECD.

The extraction of the non-ortho and mono-ortho PCBs from the samples was achieved by saponification. The wet sample was transferred to a glass bottle containing ethanol and KOH (40%). The internal PCB standards (IUPAC nos. ^{13}C-77 and ^{13}C-labelled 126) were added to this bottle and refluxed for 6 h at a temperature of 60 °C. Then, the PCBs were extracted from this solution by adding n-pentane (3 times, 50, 25, 25 ml). The n-pentane extract was washed with demiwater three times. Then, the n-pentane extract was evaporated to 10 ml in a Kuderna Danish evaporator. The extract was further evaporated to 1 ml under nitrogen gas. Then, a multilayer glass column (25 cm $\times$ 11 mm ID) filled with 5% water-deactivated basic alumina oxide (2 g) and 33% H_2SO_4-deactivated silica (4 g) was used to further purify the concentrated extract. The purified extract that was obtained from the column, was again evaporated to 1 ml under a stream of nitrogen gas. To separate interfering pesticides from the PCBs, this extract was added to a 5% water-deactivated silica column (3 g). The extract obtained from the column was evaporated to 50 μl. To separate the non-polar from the planar PCBs, the 50 μl concentrate was subjected to HPLC fractionation (PYE column), as described by Haglund et al. (1990) and Leonards et al. (1994). The sample was separated into three fractions using the HPLC; a di-tetra ortho, mono-ortho and a non-ortho fraction. The fraction containing non-ortho PCBs was evaporated to 0.5 ml. Then, 2 ml isooctane and 0.1 ml internal standard (PCB143) were added and the mixture was evaporated to 25 μl. This extract was analysed by GC-ITD. A volume of 2 ml isooctane was added to the di-tetra ortho and mono-ortho fractions, which were then evaporated to 0.2 ml. After adding the internal standard (PCB198), the extract was analysed using GC-ECD.

Bat analysis

On three locations outside the Biesbosch dead Pond bats collected. One sample contained four juvenile bats from a nursing roost of the less polluted area of Tjerkwerd, one juvenile was collected from a nursing roost of the more polluted area of Andijk and one adult was found in a hibernaculum in Wassenaar. There was no indication of the quality of the hunting area of the latter bat, but as discussed later, it is very likely that this area was less polluted than the Biesbosch.

The three bat samples were analysed for metals and PCBs using the same method as used in the analysis of the one by adult chironomid. For the analyses whole bats were used. The sample containing four bats was homogenised before the chemical analysis was made.

Results

Preview

A reliable and consistent study of the transfer of contaminants from the sediment to the adult chironomids can only be made if the bulk of the adult chironomids present in the samples have lived in or on the sediment as larvae. Further, they must have had a diet

Table 1. Chironomid species found in the samples of larvae and adults in 1993. Food- and habitat characteristics, after Moller Pillot (1984 a+b) and Moller Pillot and Buskens (1990)

Species	Larvae	Adults	Habitat	Food
TANYPODINAE				
Procladius spec.	*		sediment	
Procladius choreus		*	sediment	
Procladius (Psilotanypus) rufovittatus		*	sediment	
ORTHOCLADIINAE				
Cricotopus sylvestris		*	plant, sediment	dead algae, detritus
Limnophyes spec.		*	bank	detritus, microbes
Nanocladius distinctus		*	sediment	detritus
Paracladius spec.		*	sediment	detritus, diatomee, plant
Prodiamesa spec.	*		sediment	detritus, plant
CHIRONOMINAE, CHIRONOMINI				
Camptochironomus tentans		*	sediment	detritus
Chironomus spec.		*	sediment	detritus
Chironomus muratensis	*		sediment	detritus
Chironomus plumosus	*		sediment	detritus
Dicrotendipes spec.	*	*	sediment	detritus
Dicrotendipes nervosus		*	sediment, stone	detritus
Endochironomus cf impar			plant, sediment	(dead) plant
Einfeldia spec.	*		sediment	detritus
Glyptotendipes spec.	*		sediment	organic material
Glyptotendipes signatus		*	on bryozoans	organic material
Harnischia group		*	sediment	carnivore
Harnischia curtilamellata		*	sediment	carnivore
Microchironomus tener		*	sediment	
Parachironomus arcuatus		*	plant,stone	carnivore, org. material
Parachironomus frequens sensu Pinder		*	on bryozoans	detritus
Phaenopsectra flavipes		*	sediment	
Polypedilum spec.	*		sediment	
Polypedilum nubeculosum		*	sediment	
Stictochironomus spec.	*		sediment	detritus
CHIRONOMINAE, TANYTARSINI				
Cladotanytarsus spec.		*		
Cladotanytarsus nigrovittatus		*		

that was part of or present within the sediment, such as detritus. As can be seen in Table 1, most of the sampled adult chironomid species lived as larvae in or on the sediment (Table 1). However, *Cricotopus sylvestris*, *Endochironomus cf impar*, *Glyptotendipes signatus*, *Parachironomus arcuatus* and *Parachironomus frequens sensu Pinder* larvae live on or in other substrates. Of this list of species, only *Cricotopus* adults were caught in large numbers, but since they have very small biomass compared to other species, their presence in the samples could be neglected.

Most of the chironomids present in the samples feed on detritus. Herbivore and carnivore species were caught in small numbers and could also be neglected. To compare concentrations of contaminants found in the sediment and in larval and adult chironomids was therefore realistic.

Chemical analysis of the samples

The results of the chemical analysis of the sediment samples and the larval and adult chironomids collected in the Biesbosch are described in Table 2. The data are presented in geometric means with a 95% confidence

Table 2. Geometric mean and 95% confidence interval (C.I.) of contaminant concentrations in sediment ($N = 3$), chironomid larvae ($N = 3$) and adult chironomids ($N = 3$) collected in the Biesbosch

	Sediment	95% C.I.	Larvae	95% C.I.	Adults	95% C.I.
	mg kg^{-1} dw		mg kg^{-1} dw		mg kg^{-1} dw	
Cadmium	11.5	3.53–37.7	3.30	2.16–5.03	1.24	0.78–1.98
Mercury	1.55	1.07–2.24	0.10	0.037–0.260	0.10	0.06–0.17
Copper	75.4	43.9–130	39.0	30.6–49.7	24.1	19.2–30.3
Nickel	31.7	23.7–42.4	ND	–	ND	–
Lead	162	79.5–330	4.69	1.31–16.8	0.38	0.11–1.34
Zinc	899	528–1530	291	275–309	273	148–504
Chromium	82.6	64.2–106	1.31	0.427–3.99	0.31	0.10–0.92
	mg kg^{-1} OM		µg kg^{-1} fw		µg kg^{-1} fw	
Benzo(a)anthracene	11.8	7.97–17.3	125	52.5–298	66.7	61.8–71.9
Benzo(ghi)pyrene	13.4	7.94–22.6	243	152–388	50.7	12.1–212
Benzo(a)pyrene	13.3	7.89–22.6	52.7	36.5–76.1	28.9	17.7–47.0
Phenanthrene	11.9	9.13–15.5	301	38.4–2360	157	23.9–1040
Indeno(1,2,3,c,d)pyrene	12.0	7.52–19.3	740	253–2170	310	288–334
Pyrene	20.0	11.9–33.6	2013	1300–3120	465	431–501
Dibenzo(a,h)anthracene	ND	–	ND	–	ND	–
Anthracene	4.86	3.28–7.19	22.2	8.21–59.9	13.8	6.10–31.3
Benzo(b)fluoranthene	20.9	13.5–32.4	323	75.0–1390	66.7	61.8–71.9
Benzo(k)fluoranthene	7.81	4.15–14.7	103	22.6–469	17.5	3.36–91.4
Chrysene	15.1	12.5–18.2	338	46.7–2450	47.8	9.51–240
Fluoranthene	22.5	11.0–46.3	2930	2230–3850	730	678–787
Naphtalene	–	–	2.88	1.43–5.83	5.93	4.22–8.34
Acenaphtylene	–	–				
Acenaphtene	–	–	ND	–	ND	–
Fluorene	–	–	2.52	1.12–5.66	1.59	0.31–8.030
					μ/kg fw	
	μg kg^{-1} OM		μg/kg fw			
Pentachlorobenzene	–	–	24.0	22.6–25.6	2.31	0.34–15.9
Hexachlorobenzene	118	24.5–571	119	67.2–212	43.2	12.1–155
PCB28	149	106–208	84.0	45.1–156	10.8	1.10–106
PCB52	234	78.3–698	505	273–935	297	97.9–902
PCB74	–	–	151	50.4–451	171	121–241
PCB60	–	–	232	150–357	214	114–401
PCB101	320	138–742	502	271–927	429	168–1100
PCB118	117	35.9–380	231	97.1–550	230	126–420
PCB138	424	281–639	803	501–1290	812	422–1570
PCB153	403	242–671	1031	588–1810	1150	603–2200
PCB180	255	109–600	1467	1030–2090	1816	1360–2420
Σ7 PCB	1960	1350–2850	4656	3090–7010	3008	922–9820
PCB77	–	–	12.5	4.18–37.3	21.0	9.62–46.0
PCB126	–	–	1.34	0.324–5.58	2.25	1.35–3.72
PCB169	–	–	ND	–	ND	–
PCB123	–	–	7.08	3.10–16.2	10.2	4.98–21.0
PCB114	–	–	20.6	9.33–45.4	14.2	9.07–22.2
PCB105	–	–	176	143–215	199	114–347
PCB167	–	–	28.1	21.8–36.2	42.0	32.6–54.1
PCB156	–	–	236	208–269	306	183–511
PCB157	–	–	13.7	11.9–15.6	16.4	8.80–30.6
PCB189	–	–	3.24	2.61–4.01	1.34	0.18–10.2

Table 2. Continued

	Sediment	95% C.I.	Larvae	95% C.I.	Adults	95% C.I.
	mg kg^{-1} dw		mg kg^{-1} dw		mg kg^{-1} dw	
2,3,7,8-TCDD-eq	–	–	0.21	0.09–0.49	0.32	0.18–0.55
Dieldrin	ND	–	ND	–	ND	–
Endrin	ND	–	ND	–	ND	–
o,p′-DDE	–	–	ND	–	ND	–
p,p′-DDE	–	–	178	117–272	197	113–343
o,p′-DDD	–	–	32.8	24.3–44.4	25.9	0.86–781
p,p′-DDD	–	–	217	90.2–521	99.6	18.2–544
o,p′-DDT	–	–	ND	–	ND	–
p,p′-DDT	–	–	ND	–	ND	–
α-hexachlorocyclohexane	ND	–	52.7	31.1–89.3	53.1	42.2–66.9
β-hexachlorocyclohexane	52.5	1.15–2400	ND	–	ND	–
γ-hexachlorocyclohexane	ND	–	158	40.5–617	35.9	13.5–95.8
Heptachlor	ND	–	ND	–	ND	–
Octachlorostyrene	–	–	32.7	19.2–55.8	32.9	12.3–88.0

Σ7 PCB = PCB28+52+101+118+138+153+180; 2,3,7,8-TCDD-eq. after Van Zorge et al. (1989), dw = dry weight; fw = fat weight; ND = not detectable; OM = organic matter.

interval. The data obtained from the sediment analysis are standardised to 10% organic matter and 25% silt, in the same way as described earlier.

The actual amount of organic matter present in the sediment was 6.39±2.95%. The luteum fraction was 18.41±8.04%.

Transfer of contaminants from the sediment to chironomid larvae

The Hg, Ni, Pb and Cr concentration ratios, as measured in larvae dry weight and in standard sediment dry weight varied from 0.02 to 0.06 (Table 3). Transfer ratios for Cd and Zn were approximately 0.3; the ratio for Cu was 0.5.

Hexachlorobenzene concentrations in larvae fat were equal to those found in dry organic matter of the sediment (Figure 2a). The standard PCBs' (IUPAC: 28, 52, 101, 118, 138, 153, 180) mean concentration ratio has a value of 2.4 kg^{-1} fw/kg^{-1} dw OM. Within the standard PCBs, the higher congeners have a higher accumulation ratio than lower PCBs. The highest accumulation ratio (18.1) was observed for γ-HCH.

The transfer ratio of PAHs in juvenile chironomids varied between 0.004 (benzo(a)pyrene) and 0.13 (fluoranthene) (Figure 2b), which is clearly below the levels for persistent organochlorines.

Transfer of contaminants from larvae to adult chironomids

The fraction of heavy metals which was transferred from the larval to the adult stage varies between 0.08 for Pb to 1.0 for Hg, on a dry weight basis (Table 3).

Transfer ratios of the more persistent organics (based on fat weight) are demonstrated in Figure 2c. The geometric means of the ratios for standard PCBs (IUPAC: 28, 52, 101, 118, 138, 153, 180) and mono-ortho PCBs are approximately 1. PCB28 has a deviating ratio of 0.13. The ratio of the non-ortho PCBs (77 and 126) was 1.7. PCB169 was not measured above the detection limit.

P-p′DDE, o-p′DDD, α-HCH and octachlorostyrene were found in comparable concentrations in larval and adult chironomids. The ratio for γ-HCH was 0.2. Other pesticides were not present above the detection limit.

PAHs had an accumulation ratio ranging from 0.2 to 0.6 (Figure 2d). Only naphthalene seems to be better transferable, having a ratio of 2.

Chemical analysis in bats

Results obtained from the chemical analysis of the bats are described in Table 5. The heavy metal concentrations are based on dry weight and the PCB and pesticide concentrations are relative to the fat concentrations present in the bat samples. Fat concentration varied from 1.3 to 24.3% based on dry weight, depend-

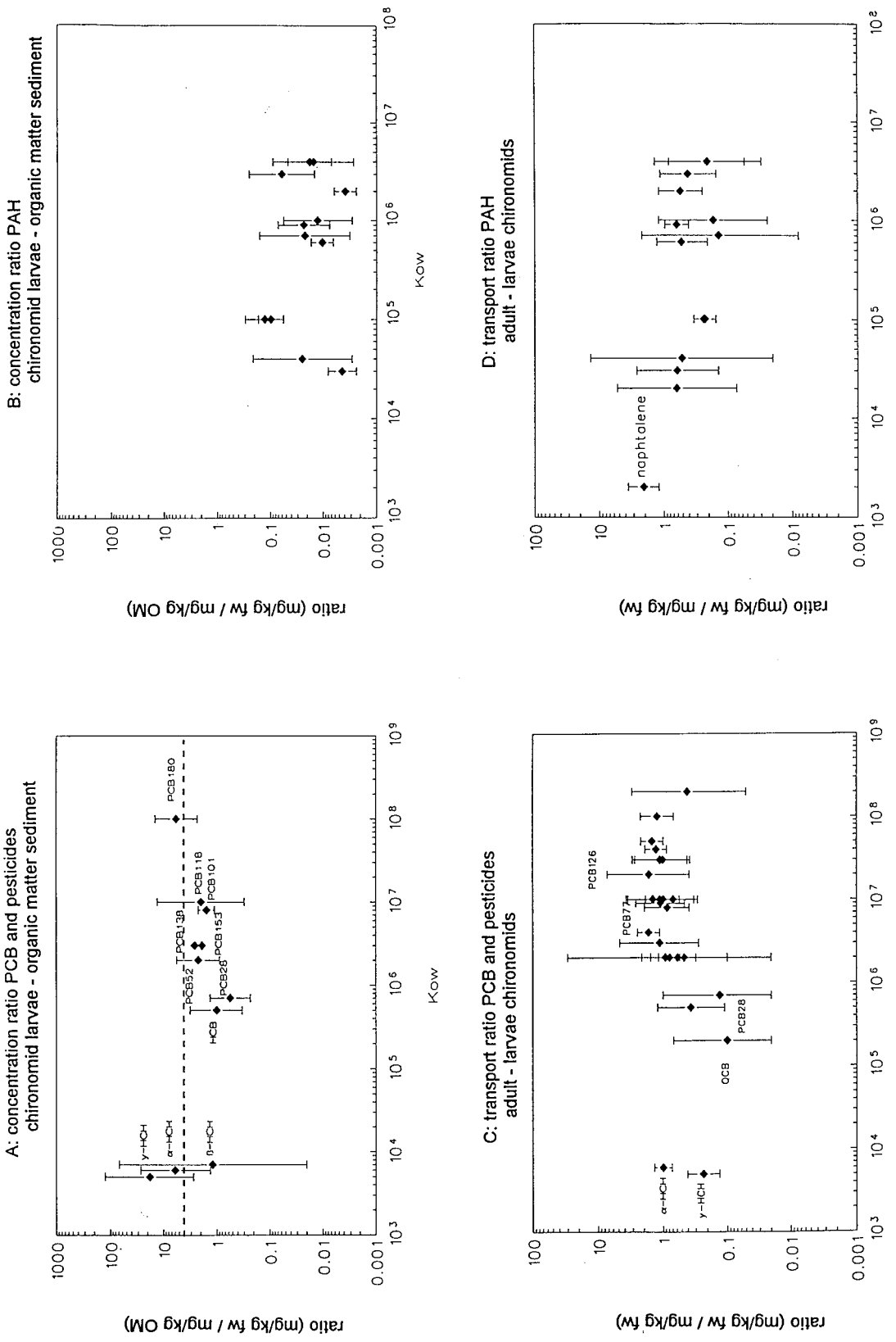

Figure 2. Accumulation or transfer ratios for microcontaminants at the Biesbosch. A and B: Chironomid larvae fat to organic matter dry weight ratios ($\mu \pm 95\%$ C.I.). C and D: Chironomid adult fat to chironomid larvae fat ($\mu \pm 95\%$ C.I.) Kow = Octanol-water partition ratio. Describes the distribution of substances between octanol (as an equivalent of biological fat) and water. Fw = fat weight, OM = organic matter.

Table 3. Concentration ratio of microcontaminants between Chironomid larvae and sediment, and between juvenile and adult chironomids

	Geometric mean ratio larvae-normalized sediment ($N = 3$) (kg^{-1} dw/kg^{-1} dw)	95% C.I.	Geometric mean ratio adult-larvae ($N = 3$) (kg^{-1} dw/kg^{-1} dw)	95% C.I.
Cadmium	0.2861*	0.06–1.41	0.3763*	0.23–0.61
Mercury	0.0636*	0.02–0.24	1.0298*	0.61–1.75
Copper	0.5165*	0.24–1.13	0.6187*	0.49–0.79
Nickel	0.0373	0.03–0.05	–	–
Lead	0.0289*	0.0088–0.095	0.0811	0.067–0.098
Zinc	0.3242*	0.20–0.53	0.9384*	0.49–1.81
Chromium	0.0158*	0.0046–0.054	0.2317	0.027–1.98

*All values > detection limit.
−5 to 6 values < detection limit.

ing on the time of the year when the bats died. Fat concentrations strongly increase towards the winter and decrease during hibernation.

Discussion and conclusions

Sediment

In order to determine the reliability of the Biesbosch measurements, these data were compared to data obtained from the Hollandsch Diep. Since the Hollandsch Diep is adjacent to the Biesbosch and also a part of the Rhine-Meuse Delta, the results from both locations should be comparable.

Most of the contaminants measured in our sediment samples appeared in equal or higher concentrations in the Hollandsch Diep (Hendriks, 1995).

The concentrations of Cd, Cr, Hg Pb and Zn measured in the Biesbosch samples were comparable to those found in the Hollandsch Diep. HCHs were also found in equal levels at both locations. Ni levels measured in the Hollandsch Diep, as well as the sum of the seven standard PCBs, were three times those found in the Biesbosch, the hexachlorobenzene concentration was four times that of the Biesbosch.

Cu levels at the Hollandsch Diep were three times lower than those of the Biesbosch, making Cu the only contaminant -in this study- that had higher concentration levels in the Biesbosch.

Data of PAHs of the sediment of the Hollandsch Diep are not available for comparison.

Chironomid larvae and adults

Pb and Cu concentrations were three times lower in the chironomid larvae collected from the Biesbosch than those from the Hollandsch Diep. Zn levels were six times lower in the Biesbosch larvae. In contrast, the cadmium concentration found in the Biesbosch larvae was four times higher than that of the Hollandsch Diep larvae (Van Hattum et al., 1993). Concentrations of the 7 standard PCBs and the hexachlorocyclohexanes in Chironomid larvae are comparable to those measured in the Hollandsch Diep by Hendriks & Pieters (1993).

Data on adult chironomids from the Hollandsch Diep were not available.

Larvae:sediment ratio

In general, the concentration ratios for the metals are similar to the values measured by Van Hattum et al. (1993) for another part of the Rhine-Meuse delta. Zn, Cu and Cd have the highest transport ratios, ranging from 0.3 to 0.5 (Table 3). The transfer ratios of Cr, Ni, Pb and Hg are approximately 10 times lower.

In general, the 95% confidence intervals of the PCB and pesticide ratios include the 3.3 value derived from laboratory studies (Hendriks, 1995). These results are also in accordance with the results obtained from field studies on oligochaetes (Ankley et al., 1992; Bierman, 1990). We can therefore conclude that an average transfer ratio of 3.3 for PCBs and pesticides is a reliable value, which has been derived from field experiments as well as laboratory studies, and is applicable to chironomids and oligochaetes.

Table 4. Results of laboratory studies on effects of microcontamination on bats.

	Conc. in food mg kg^{-1} ww	Conc. in bat mg kg^{-1} fw	Number of animals	Number of dead young (y) or dead adults (a)	Exposer time (day)	Reference
AROCLOR 1260						
Eptesicus fuscus	0.08	45	18 pregnant ♀	5 y out of 36	22.8	Clark, 1978
Eptesicus fuscus	6.4	3.8×10^{2}°	18 pregnant ♀	1 y out of 37	22	Clark, 1978
Myotis lucifugus	0.56	2.1×10^{2}°	7	0 a	40	Clark & Stafford, 1981
Myotis lucifugus	15	32×10^{2}°	12	2 a	40	Clark & Stafford, 1981
Myotis lucifugus	10×10^{2}	17×10^{4}	5	4 a	40	Clark & Stafford, 1981
AROCLOR 1254						
Eptesicus fuscus	–	11°	5	0 a	37	Clark & Prouty, 1977
Eptesicus fuscus	9.4	6.6×10^{2}°	16	2 a	37	Clark & Prouty, 1977
DDE						
Eptesicus fuscus	–	65°	5	0 a	54	Clark & Prouty, 1977
Eptesicus fuscus	1.7×10^{2}	43×10^{2}°	11	0 a	54	Clark & Prouty, 1977
Myotis lucifugus	–	1.0×10^{2}°	7	0 a	40	Clark & Stafford, 1981
Myotis lucifugus	1.5×10^{2}	28×10^{3}°	11	1 a	40	Clark & Stafford, 1981
Myotis lucifugus	4.8×10^{2}	42×10^{3}	5	2 a	40	Clark & Stafford, 1981

° = death by starvation.

From literature research on the transport of PAHs in laboratory experiments, a value of 3.3 has been calculated (Hendriks, 1995). Fieldstudies on Chironomidae and Oligochaetes, in which the data of both taxa were combined, showed a transport ratio of approximately 0.3 (Hendriks, 1995).

The PAH ratios of our study vary between 0.004 and 0.1 (kg^{-1} fw/kg^{-1} dw). These data are not consistent with the field data described by Hendriks. This discrepancy can be due to a number of facts. Firstly, in both studies a small number of samples was used. Secondly, in Hendrik's study, data from two different animal taxa were combined. However, both field experiments do show a significantly smaller transport ratio than the laboratory studies.

In general, our data of the concentrations of microcontaminants in sediment and in larvae, and the transfer ratio larvae-sediment are comparable to other data of the Rhine-Meuse delta. Large variation of microcontaminant concentrations within time or within the sampling area are therefore unlikely. Over the years the microcontaminant concentrations in the Biesbosch are also very consistent. The microcontaminants are very persistent and there is now hardly any transport of (less polluted) sediment. Most of the pollutants of the Biesbosch are deposited shortly after closing the Haringvliet barrage (1970). A large area was sedimentated within a short period which explains the small differences of microcontaminant concentrations. Spots with a strongly deviated pollution degree are scarce within the Biesbosch. To collect the samples of the sediment and the larvae in two successive years (1992 and 1993) and within a radius of a few meters seems therefore acceptable.

Adult:larvae ratio

In our field experiments the transfer ratios for Pb, Cu, Cd, Zn and Hg were 0.08, 0.4, 0.6, 0.9 and 1.0, respectively. In general, this pattern is consistent with the laboratory data described in Timmermans & Walker (1989) and Kranzberg & Stokes (1988). They found that 20–30% of the essential metal Zn eliminated during the metamorphosis from larvae to adult, 70–90% of the essential and toxic metal Cu and 90% of the toxic Cd is lost.

Most of the persistent organics have comparable concentrations in both life stages (on fat weight), thus a ratio of 1.0, which is in accordance with the studies of Fairchild et al. (1992) and Larsson (1984). Fairchild et al. (1992) found mean concentrations of 2,3,7,8-TCDF of 158 and 228 pg g^{-1} wet weight in larval chironomids and adult chironomids, respectively, which gives a transport ratio of 1.4. To be able to compare this ratio with our results, we recalculated

Table 5. Concentrations of contaminants in Dutch Pond bats (*Myotis dasycneme*) found in 1993

		Andijk[1]	Tjerkwerd[2]	Wassenaar[1]
Cd	mg kg^{-1} ds	0.29	0.06	0.17
Hg	mg kg^{-1} ds	0.72	0.07	0.78
PCB 28	μg kg^{-1} fw	215	82	576
PCB 52	mug kg^{-1} fw	46	<105	26
PCB 60	μg kg^{-1} fw	317	173	<18.4
PCB 74	μg kg^{-1} fw	1520	481	1480
PCB 77	μg kg^{-1} fw	8.47	10.0	2.78
PCB101	μg kg^{-1} fw	129	106	114
PCB105	μg kg^{-1} fw	1620	171	1360
PCB114	μg kg^{-1} fw	210	176	75.2
PCB118	μg kg^{-1} fw	7820	1000	4180
PCB123	μg kg^{-1} fw	129	1105	65.8
PCB126	μg kg^{-1} fw	19.7	1208	8.06
PCB138	μg kg^{-1} fw	9050	948	6550
PCB153	μg kg^{-1} fw	29240	3120	11000
PCB156	μg kg^{-1} fw	5290	456	1820
PCB157	μg kg^{-1} fw	283	23.8	105
PCB167	μg kg^{-1} fw	1090	81.8	295
PCB169	μg kg^{-1} fw	3.54	<1.50	0.914
PCB180	μg kg^{-1} fw	18396	1740	5260
PCB189	μg kg^{-1} fw	265	15.6	68.5
Pentachlorobenzene	μg kg^{-1} fw	577	277	72.4
Hexachlorobenzene	μg kg^{-1} fw	229	<14.1	548
Heptachlor	μg kg^{-1} fw	55.9	<17.9	<4.27
Octachlorostyrene	μg kg^{-1} fw	157	<13.3	130
o-p′DDE	μg kg^{-1} fw	<8.80	<37.2	<8.88
p-p′DDE	μg kg^{-1} fw	14370	1410	3504
o-p′DDD	μg kg^{-1} fw	288	<150	156
p-p′DDD	μg kg^{-1} fw	995	<1398	276
o-p′DDT	μg kg^{-1} fw	<9.59	<40.6	<9.68
p-p′DDT	μg kg^{-1} fw	1370	272	71.8
α-HCH	μg kg^{-1} fw	486	24.3	30.0
β-HCH	μg kg^{-1} fw	536	225	124
τ-HCH	μg kg^{-1} fw	291	177	<13.1
Cis-heptachloro-epoxide	μg kg^{-1} fw	49.3	<70.7	<18.8
Trans-heptachloro-epoxide	μg kg^{-1} fw	<16.9	<70.9	<18.8
Dieldrin	μg kg^{-1} fw	537	<91.5	920
Endrin	μg kg^{-1} fw	<38.2	<160	<42.5

[1]Sample contained 1 animal.
[2]Sample contained 4 animals.
< not present above detection limit.

our transfer ratio for wet weight. This gave us a value of 1.6, which is in accordance with Fairchild's ratio. Larsson (1984) found a transfer ratio of 0.9 (kg^{-1} fw/kg^{-1} fw) for Clophen A-50 (a mixture of PCBs), which is very similar to our findings.

Three of the persistent organics have a transfer ratio higher than 1.0. These are PCB-77, PCB-126 and naphtalene. This means that higher amounts of these contaminants were found in the adults than were present in the larvae. Further experiments have to be conducted to determine the cause of this phenomenon.

Our findings are consistent with other studies. Scepsis about the representativity of the data of the adult chironomids for the sampling area seems there-

fore incorrect. The adult chironomids caught probably did not all emerge at the sampling place but at least in the direct surroundings. In the direct surroundings we assume – as discribed before – no large variation in contamination concentrations.

Risks for the Pond bat

The risks for the Pond bat in the Biesbosch can be estimated by comparing the concentrations of contaminants found in the food of the bats (adult chironomids) with 'safe' concentration levels obtained from literature. For mammals and birds, Romijn et al. (1993) and Van de Plassche et al. (1991) derived diet levels that were thought to be safe for mammals. They published safe concentrations levels of cadmium, mercury, PCB-153, hexachlorobenzene, γ-HCH, dieldrin, p-p′DDT, o-p′DDT, p-p′DDE and p-p′DDD. None of the mean concentrations of each of the contaminants found in the Biesbosch adult chironomids exceed these safe maximum levels. Kociba et al. (1978) found effects in rats fed food containing 210×10^{-3} μg kg^{-1} ww 2,3,7,8-TCDD. They found no effects at concentrations of 22×10^{-3} μg kg^{-1} ww. In adult chironomids of the Biesbosch a mean concentration of 19×10^{-3} μg kg^{-1} 2,3,7,8-TCDD-eq ww was found. Thus, the concentration levels of Cd, Hg, PCB-153, HCB, γ-HCH, dieldrin, p-p′DDT, o-p′DDT, p-p′DDE, p-p′DDD and TCDD of the Biesbosch chironomids do not seem to be a risk for the Pond bat.

Clark and others have studied the toxicity of some organics on bats (Table 4). Bats were fed contaminated mealworms *Tenebrio molitor* over a period from 22 to 54 days. The contamination effect is expressed in the number of dead animals. Aroclor 1260, Aroclor 1254 and p-p′DDE were lethal at concentrations of 15, 9.4 and 150 mg kg^{-1} ww, respectively. The concentration of PCBs found in the Biesbosch chironomids (0.3 mg kg^{-1} ww) is, according to the results described by Clark (1978), not lethal and does not cause clear sub-lethal effects. The concentration of p-p′DDE in chironomids (0.01 mg kg^{-1} ww) will not be lethal for bats according to Clark & Prouty (1977).

The sum of the concentrations of the 19 analysed PCBs for our three bat samples were 9, 33 and 76 mg kg^{-1} lipid weight. These concentration levels are equal or higher compared to the NOEL for reproduction effects of Mink *Mustela vison*: 9 mg PCB kg^{-1} lipid weight (Kihlström et al., 1992). The ΔPCB concentration in the hibernating bat ($>$65 mg kg^{-1} lipid weight) even exceeded the EC50 level for reproduction effects of Mink (50 or 65 mg kg^{-1} lipid weight), reported by Jensen et al. (1977) and Kihlström et al. (1992). Thus, if bats are as sensitive to PCBs as mink, PCB-levels in *Myotis dasycneme* are a cause for concern. The few toxicity experiments that have been conducted on bats (Table 4.) do not suggest the same level of sensitivity. However, the fact that the Biesbosch is more polluted than the known hunting area of the bats that were analysed suggest higher PCB levels in bats hunting in the Biesbosch. In general, information on bat toxicity is scarce and even effects from low concentrations of contaminants cannot be completely excluded. Future research should therefore be concentrated on the effect of contaminants on bats, both in the laboratory and under field conditions.

Recently, some studies reported that toxicity of PCBs may partly be due to the metabolites of some PCB congeners. Brouwer (1991), for instance, reported that higher mammals metabolize PCB 77, which is one of the substances that accumulate in the food chain of the bats, as reported above. The metabolite of this PCB congener interferes with the transport system of vitamin A (Brouwer, 1991). An indication of the metabolisation of PCB 77 in Pond bats can be derived by comparing the concentration levels of PCB 77 to those of PCB 153, which cannot be metabolised, in both the Pond bat and the adult chironomids. This relative concentration (PCB 77/PCB 153) found in the analysed bats was seven times lower than that of the Biesbosch chironomids (respectively 0.0031, 0.023). Therefore, it seems that PCB 77 was metabolised by Pond bats, which may contribute to toxic effects.

Acknowledgements

We would like to thank M. Davis, C. van der Guchte, H. Limpens, C. Schmidt, H. Vallenduuk, J. van der Velden, S. Wiersma for their help. We are also grateful to P. Lina, F. van der Vliet and A. Voûte, who made dead Pond bats available. For the analysis we thank I. Burgers, G.J. van Kesteren and C. Swart. For the review: B. van Hattum, P. Leonards and Y. van Vugt.

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