Species diversity and human health are constantly threatened by the production and release of toxic chemicals into the atmosphere, water, or soil. Polychlorinated biphenyls (PCBs) and chlorinated pesticides belong to a class of ubiquitous pollutants that are highly lipophilic and refractory to chemical degradation. Because of these physicochemical properties and their slow rate of enzymatic biotransformation in all organisms, such organochlorine pollutants tend to accumulate to toxic levels in both aquatic and terrestrial food chains (Eacobichon 1996; Hill 1999; Jorgenson 2001; Loizeau et al. 2001). Chronic exposure to persistent organochlorine chemicals has been associated with deleterious effects in fish and wildlife, leading to poor fertility, impaired immune functions, and population declines in the contaminated areas (Colborn et al. 1993; Fry and Toone 1981; Guillette et al. 1996; Tyler et al. 1998).

Similarly, epidemiologic studies suggest that various diseases of the reproductive system in humans, ranging from breast, endometrial, or testicular cancer to a worldwide drop of sperm number and quality, may be linked to organochlorine pollutants (Golden et al. 1998; Hunter et al. 1997; Krieger et al. 1994). Furthermore, prenatal or perinatal exposure to persistent organochlorine chemicals has been implicated as a risk factor for low birth weight, thyroid and immunologic defects, and the development of neurobehavioral deficits in pediatric cohorts (Kimbrough et al. 2001; Seegal 1994; Vreugdenhil et al. 2002; Winneke et al. 1998).

A potentially hazardous chemical becomes toxic only when it reaches an active site in the target species and is sufficiently present to concentrate to generate adverse reactions. Therefore, exposure levels in combination with toxicokinetic indices such as uptake, metabolism, and elimination are important parameters that determine the health risk resulting from a particular toxic substance. Before establishing reliable risk assessment studies, therefore, it is necessary to determine the biologically available level of toxic chemicals and their potential accumulation, that is, the efficiency by which such chemicals are transported from environmental matrices to relevant biologic receptors (Eaton and Klaassen 1996; Hutton 1982; MacKay 1991; Thibodeaux 1979). Typically, the bioavailability and bioaccumulation of environmental pollutants are assessed by measuring chemical residues in tissues and fluids of animals or humans. Many different species can be used to estimate the impact of toxic chemicals on aquatic habitats (Kendall et al. 1996; Wells 1999), but the choice of suitable terrestrial indicators is more problematic (Beeby 2001; van der Schalie et al. 1999). For example, wild mammalian species such as chamois, European hare, moose, red deer, reindeer, roe deer, wild mink, and wolves have been proposed for biomonitoring studies (Doganoc and Gacnik 1995; Frosli et al. 1986; Kottferová and Koréneková 1998; Poole et al. 1998; Santiago et al. 1998; Shore et al. 2001). Unfortunately, with few exceptions, these wildlife species are represented only in their natural habitats, including forests or other rural areas, and often occupy very large territories, such that they may not be helpful in identifying local sources of contamination that are directly relevant to the exposure level of people.

The lack of adequate terrestrial sentinel species has led previous researchers to evaluate the potential contribution of rodents, birds, soil or parasitic invertebrates, and even plants as indicator organisms for environmental monitoring studies (e.g., Beeby 2001; Cook et al. 1994; Hutton 1982; Labrot et al. 1999; Moss et al. 2001; Rodgers et al. 2001; Sures et al. 2002). A convenient alternative species that lives in close vicinity to humans is provided by the increasing colonization of suburban and urban habitats by the red fox (Vulpes vulpes). Indeed, red foxes have already been used to monitor anthropogenic pollutants in rural habitats (Ansorge et al. 1993; Brunn et al. 1991; Bukovjan 1997; Corsolini et al. 2000; Georgi et al. 1994). Being a highly opportunistic species that adapts to a multitude of different environmental conditions and food supplies (Nowak 1991), red foxes have progressively invaded the cities and suburbs of many European countries (Schöffel et al. 1991; Willingham et al. 1996), where these animals are exposed, at least in part, to the same pollutants and contaminants as the local human population. A similar development also...
took place in other parts of the wide distribution area of red foxes, including Canada and Japan (Gloor et al. 2001). During recent years, the fox population in Zürich, the largest city of Switzerland, increased to a density of 6.9–10.3 adult foxes/km², with cubs being reared in public parks and in private gardens (Hofer et al. 2000). Contrary to the typically large territories encountered in rural habitats, the home range of these urban foxes is limited to small areas of ≤ 0.5 km² (Doncaster and Macdonald 1991; Harris 1997; Harris and Trewella 1988), so the geographic area being monitored by the analysis of each animal is well defined. The recent finding that red foxes collected from separate sites in the city of Zürich contain distinctly different levels of toxic metals (Dip et al. 2001) confirmed that the composition of chemicals in their organs is representative of locally occurring pollutants or contaminants. For example, mean lead concentrations were nearly three times higher in the liver of juvenile foxes from the urban center than in the corresponding tissue of their rural counterparts. In this study, we analyzed the distribution of organochlorine pollutants in the fox population living in the city of Zürich and its suburbs as a first step to validate this possible sentinel species against the known human exposure and, hence, demonstrate its significance with respect to health hazards in people. Our findings indicate that the analysis of urban and suburban foxes may not only serve to estimate the magnitude of environmental pollution but may also help to identify persistent chemicals that pose a particular threat to sensitive human risk groups.

Materials and Methods

Foxes. Red foxes were collected between January 1999 and February 2000 in Zürich and its suburbs, which are inhabited by about a million people. This study area consists of an urban and suburban center of 60 km², surrounded by forests and farmland amounting to 30 km². Most foxes (~75%) were shot by local game wardens; the remaining animals were killed during road or traffic accidents (no animals were killed for the purpose of this study). The exact coordinates of each collection site were recorded. The carcasses were wrapped in plastic bags and stored at −20°C until necropsy. Tissues were carefully examined for postmortem degradation and for the presence of shots or shot injuries, and, if present, such samples were eliminated from further analysis. The age of each animal was determined by counting annuli in the cementum of a canine tooth of the lower jaw (Harris 1978).

Sample analysis. Organochlorine concentrations were assessed in samples of homogenized kidney using a validated procedure that follows the method by Corsolini et al. (2000) with several modifications. Samples were extracted in the presence of a mixture of n-hexane and dichloromethane at a ratio of 4:1. The apolar phase was processed through two consecutive Florisil columns eluted with n-hexane/dichloromethane at ratios of 4:1 on the first column and 9:1 on the second column. Analyses were performed with an HRGC 5160 gas chromatograph (Carlo Erba, Milan, Italy) equipped with 63Ni electron capture detector and a fused silica capillary column (60 m × 0.25 mm × 0.25 µm) (MSP-Friedli, Koeniz, Switzerland). The carrier gas was hydrogen at 2.0 mL/min with a splitting ratio of 15:1. The temperatures of the injector and detector were 260°C and 315°C, respectively. The oven was kept at 100°C, and then increased to 160°C at 10°C/min, then to 265°C at 3°C/min, and finally set at 295°C (with an increase of 20°C/min). Pure reference standards (Dr. Ehrenstorfer, Augsburg, Germany) were used for recovery determination and quantification. Each series of analysis included a blank, a standard calibration curve, and spiked specimens. Recovery of organochlorines with spiked samples ranged from 80% to 100%. In the case of dieldrin and endrin, recovery was reduced to about 40%. All results are given in milligrams per kilogram on a wet-weight basis and represent arithmetic means ± standard error of the mean (SE). The coefficient of variation on replicate probes was < 7%. The limit of detection in tissue samples was 0.005 mg/kg.

Statistics. PCB, dieldrin, and dichlorodiphenyltrichloroethane (DDT) concentrations between different areas, age groups and sexes were compared by the Mann-Whitney U test and the Kruskal-Wallis nonparametric analysis of variance (ANOVA) using the Graphpad Instat version 3.0a software (Graphpad, San Diego, CA, USA). The Kruskal-Wallis posttest for multiple comparisons according to Dunn was applied to determine p values, and differences were considered significant at p < 0.05.

Results

Composition of sample population. A sample of perirenal adipose tissue was obtained from a total of 192 red foxes collected between January 1999 and February 2000. Fifty-nine animals were from the urban center of Zürich; 122 animals were collected in the suburban area, and only 11 foxes were from the near rural surroundings (Figure 1). Upon age determination, these animals were divided into three groups: juvenile foxes ≤ 12 months of age (n = 108), young foxes between 13 and 24 months of age (n = 36), and adult foxes > 2 years of age (n = 48). The study population consisted of 47% females and 53% males.

Organochlorine residues. A series of persistent organochlorine pollutants were determined by gas chromatography coupled to electron capture detection. The quantitatively most important residue fraction consisted of PCBs, a class of ubiquitous environmental pollutants comprising 209 possible congeners with different chlorine substitution patterns (Giesy and Kannan 1998). These individual congeners have different physicochemical properties that influence their bioavailability, metabolism, and half-life such that, in most biologic extracts, PCB-138, PCB-153, and PCB-180 constitute the dominating components (Coglano 1998; Safe 1994). These major PCB congeners were detected in the adipose tissue of all 192 foxes, although at relatively low levels (Table 1). PCB-138 was present at concentrations ranging between 0.005 mg/kg (the limit of detection) and 0.150 mg/kg. The lowest PCB-153 and PCB-180 levels were around 0.010 mg/kg. The highest concentrations observed for PCB-153 and PCB-180 were 0.485 and 0.357 mg/kg, respectively.

| Positive samples (%) | Maximum (mg/kg) | Mean² (mg/kg) |
|----------------------|----------------|---------------|
|                      | U              | S             | R              | U             | S             | R              | U             | S             | R             |
| ΣPCB (138, 153, 180) | 100            | 100           | 100            | 0.939         | 0.790         | 0.266         | 0.221         | 0.199         | 0.165         |
| PCB-28               | 0              | 1.7           | 0              | < 0.005       | 0.006         | 0.005 NA²      | 0.006 NA²     | 0.005 NA²     | 0.006 NA²     |
| PCB-101              | 15.3           | 5.8           | 0              | 0.098         | 0.011         | < 0.005       | 0.015         | 0.007         | 0.007         |
| Dieldrin             | 72.9           | 70.5          | 54.5           | 0.112         | 0.154         | 0.113          | 0.016         | 0.023         | 0.018         |
| Endosulfan           | 35.6           | 34.4          | 18.2           | 0.063         | 4.381         | 0.010          | 0.021         | 0.019         | 0.009         |
| Hexachlorobenzene    | 39.0           | 18.2          | 9.1            | 0.027         | 0.040         | 0.006          | 0.010         | 0.009         | 0.005         |
| Heptachloroepoxide   | 50.8           | 45.5          | 27.3           | 0.015         | 0.127         | 0.060          | 0.006         | 0.007         | 0.006         |
|                      | 5.1            | 14.1          | 9.1            | 0.008         | 0.013         | 0.007          | 0.007         | 0.006         | 0.007         |

Abbreviations: NA, not applicable; R, rural area; S, suburban; U, urban.

*Mean values of positive samples; the results under the limit of detection were not included. **Mean value could not be calculated because all samples were below the limit of detection.
respectively. In contrast to these major congeners, 138, 153, and 181, which were detected in all animals, PCB-28 was found above the limit of detection of 0.005 mg/kg in only two foxes. All foxes resulted negative for PCB-52, but PCB-101 was found in 17 animals at concentrations of 0.005–0.011 mg/kg. Similarly, detectable concentrations of organochlorine insecticides were encountered only in a fraction of the animals. For example, 70% of tissue samples were positive for dieldrin with concentrations of up to 0.154 mg/kg. Similarly, 34% of the samples resulted positive for DDT with the highest concentration reaching 4.361 mg/kg. Only 24% of the animals contained trace levels of endosulfan, 46% were positive for hexachlorobenzene, and heptachlor epoxide could be detected in 11% of the adipose tissue samples (Table 1).

Comparison between foxes from different habitats. In a previous study with the same fox population (Dip et al. 2001), we found that animals from adjacent but different environmental compartments contained distinct levels of heavy metals. These previous results confirmed that urban foxes really occupy their city habitats and are not just occasional roamers from the surrounding rural areas. Here, we observed a gradient of PCB concentrations in adipose tissue (sum of PCB-138, PCB-153, and PCB-180) from the urban foxes to the animals living in the suburbs and rural surroundings (Table 1). In particular, PCB-138 was preferentially accumulated in foxes of the urban center (Figure 2A). The increased mean value for PCB-138 in urban foxes relative to the rural animals was significant upon analysis by the Mann-Whitney U test. An apparently similar but statistically not significant trend was observed for PCB-153. No regional difference was detected in the level of congeners PCB-101, DDT, and endosulfan (see “Materials and Methods” for details). No such general decrease could be detected for organochlorine insecticides, because there was a very similar dieldrin concentration in all three age groups (Figure 2B). Also, the content of PCB-28, PCB-101, DDT, endosulfan, hexachlorobenzene, and heptachlor epoxide was not significantly different in the three age groups. Nevertheless, it is remarkable that the animals with the highest concentrations of PCB-28 (0.006 mg/kg), PCB-101 (0.089 mg/kg), dieldrin (0.154 mg/kg), DDT (4.361 mg/kg), endosulfan (0.027 mg/kg), and hexachlorobenzene (0.033 mg/kg) always belonged to the category of juvenile foxes.

Age-dependent distribution of organochlorine residues. The entire fox population, including the small rural fraction of only 11 animals collected at the city border, was further dissected according to age (Table 2). We calculated an additive PCB concentration (sum of PCB-138, PCB-153, and PCB-180) of 0.235 ± 0.018 mg/kg (mean ± SE) in juvenile foxes ≥ 12 months of age. Surprisingly, this total PCB concentration decreased to 0.146 ± 0.016 mg/kg in the adult animals > 24 months of age. Notably, the absolutely highest PCB load (sum of PCBs amounting to nearly 1 mg/kg) was detected in a juvenile fox, whereas the absolutely lowest sum of PCBs (0.019 mg/kg) was found in an adult fox. The same general trend of reduced PCB burden with increasing age was observed with all individual congeners tested (Figure 2B). In all of these cases, the overall difference between juvenile and adult animals appeared significant upon data analysis by both the Mann-Whitney U test and nonparametric ANOVA (see “Materials and Methods” for details). No such general decrease could be detected for organochlorine insecticides, because there was a very similar dieldrin concentration in all three age groups (Figure 2B). Also, the content of PCB-28, PCB-101, DDT, endosulfan, hexachlorobenzene, and heptachlor epoxide was not significantly different in the three age groups. Nevertheless, it is remarkable that the animals with the highest concentrations of PCB-28 (0.006 mg/kg), PCB-101 (0.089 mg/kg), dieldrin (0.154 mg/kg), DDT (4.361 mg/kg), endosulfan (0.027 mg/kg), and hexachlorobenzene (0.033 mg/kg) always belonged to the category of juvenile foxes.

Sex-dependent distribution of PCB residues. When the distribution of PCBs was further analyzed by separating the foxes according to their sex, we observed only slightly higher PCB levels in males than in females. However, a clear difference between the two sexes was evident after plotting the concentration in each group of animals as a function of age (Figure 3). Male foxes showed significantly higher PCB concentrations in all age groups compared to females (Figure 3).

| Figure 2. Mean concentration (± SE) of PCBs and dieldrin in the adipose tissue of foxes. (A) Comparison between different environmental compartments. (B) Comparison between different age groups. *p < 0.05 for differences between urban and rural foxes. **p < 0.05 for differences between juvenile and adult foxes. #p < 0.05 for differences between juvenile and young foxes. | Figure 3. PCB concentrations (mean ± SE) in the adipose tissue of foxes by sex. (A) Sum of the three major congeners (PCB-138, PCB-153, PCB-180). (B) PCB-153. *p < 0.05 for differences between juvenile and adult foxes. |
|---|---|
| Table 1. Comparison between foxes from different age groups. | Table 2. Results of organochlorine analysis by age group. |
of age. In fact, a statistically significant (Mann-Whitney U test and ANOVA) reduction of PCB concentration was found in the adipose tissue of female foxes, where the sum of PCBs decreased from 0.225 ± 0.032 mg/kg (mean ± SE) in juvenile animals to 0.129 ± 0.018 in adults (Figure 3A). In contrast, the male foxes maintained approximately the same PCB level in the fatty tissue through all stages of life (Figure 3A). The age-dependent decrease of PCB burden in female animals was significant for all individual PCB congeners tested, but the difference between juvenile and adult animals was particularly strong with PCB-153 (Figure 3B). In contrast, the PCB-153 content of adult males was not significantly different from that of juvenile animals (Figure 3B). The overall higher PCB concentration in juvenile foxes relative to adults, in combination with the selective drop of PCB levels in females, indicates that these compounds are transported from mothers to offspring during pregnancy or lactation. This elimination mechanism is absent in male foxes.

Discussion

Red foxes living in urban areas were first reported in Great Britain, where the phenomenon has been observed in cities such as London since the 1930s (Teagle 1967). Since then, the urbanization of red foxes has become a trend in many cities across the globe, where rich anthropogenic food resources can sustain high fox population densities. A recent analysis of 402 urban foxes around Zürich revealed that more than half of the average stomach content of these foxes came from anthropogenic sources, that is, food scraps and garbage (Contesse et al. in press). This finding is particularly relevant when considering that diet can be the major route of exposure to polychlorinated pollutants than did that of the respective mothers (Abraham et al. 1996, 1998). Thus, an exceptional exposure to persistent organochlorine pollutants occurs in people during a critical stage of postnatal development, and a similar pattern is observed in urban and suburban foxes. This comparison suggests that the red fox or other urban wildlife species such as martens or badgers, sharing the same environment and in part the same diet with the human population, may be employed to monitor the bioavailability, accumulation, and distribution of persistent chemicals. Although such sentinel species data cannot be used as the sole factor in evaluating human health concerns, they provide additional monitoring strategies that should be particularly useful when studies on human volunteers are not practicable and the available information is therefore insufficient for a comprehensive risk assessment. For example, the analysis of urban and suburban fox populations may contribute to the early identification of newly released chemical hazards that rapidly accumulate in children or other sensitive risk groups.

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