Environmental Research 112 (2012) 77–82

Contents lists available at SciVerse ScienceDirect

Environmental Research

journal homepage: www.elsevier.com/locate/envres

Testing for bias in a sentinel species: Contaminants in free-ranging domestic, wild, and hybrid mink

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ARTICLE INFO

Article history:
Received 18 July 2011
Received in revised form
5 October 2011
Accepted 7 November 2011
Available online 30 November 2011

Keywords:
Hybridization
American mink
Contamination
Bias
Domestication

ABSTRACT

Sentinel species are important tools for studies of biodiversity and environmental health. The American mink (*Neovison vison*) has long been considered a sentinel of environmental contamination, since the species is known to be sensitive to a number of common contaminants, including polychlorinated biphenyls (PCB) and mercury. Mink may not always satisfy an important criterion of sentinels however—that they are continuous residents of the environment being sampled. This is because domestic mink commonly escape from farms, and can be confused with wild mink in areas where mink ranching is prevalent, biasing estimates of environmental contamination taken from free-ranging mink samples. We tested for bias in a sample of free-ranging mink from Ontario, Canada, where both genetic ancestry (domestic, wild, and domestic–wild hybrid) and contaminant burdens (PCBs and mercury) were known. Of 133 mink sampled for both contaminants and genetic ancestry, 9% were determined to be domestic and 10.5% hybrid animals. We found that including domestic and hybrid mink in our analysis resulted in overestimating mean PCB burdens in wild mink by 27%, and underestimating mercury by 13%. We also investigated morphological methods to aid in excluding domestic mink from free-ranging mink samples and found that we had the highest classification success using skull size (condylobasal length), which was 15% and 12% greater in male and female domestic than wild mink, respectively. Given the potential use of mink as sentinels, and also the potential for bias, we recommend that researchers take steps to exclude domestic mink from free-ranging mink samples in studies of environmental health.

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1. Introduction

American mink (*Neovison vison*) were discovered in the 1970s to be sensitive to environmental contaminants such as polychlorinated biphenyls (PCB) and mercury (e.g., Aulerich and Ringer, 1977). Since then, mink have been used as a sentinel species in studies of environmental health (e.g., Martin et al., 2006; Poole et al., 1998; Proulx et al., 1987; Wren, 1991; Yates et al., 2005) because they meet several criteria considered important for sentinels (Basu et al., 2007, 2009).

One criterion for sentinel species that is of particular importance for mink is the need for sentinels to be continuous, lifetime residents of the environment being monitored (Landres et al., 1988; Bowman and Schulte-Hostedde, 2009). This criterion is relevant for mink because it has been repeatedly shown that domestic mink escape from mink farms, which means that samples of mink used to assess environmental health may contain domestic escapees (Bowman et al., 2007; Kidd et al., 2009; Zalewski et al., 2010). Furthermore, escaped domestic mink may mate with wild mink, leading to domestic–wild hybrids and introgressed individuals (Kidd et al., 2009). The probability of encountering domestic and hybrid individuals in studies of wild mink can be quite high. For example, 64% of free-ranging mink genotyped in a recent study in southern Ontario, Canada were either domestic or domestic–wild hybrids (Kidd et al., 2009).

Domestic mink samples could well have contaminant burdens that reflect farm environments rather than natural environments, leading to biased estimates of environmental health (Bowman and Schulte-Hostedde, 2009). The extent of bias would depend on a number of uncertainties that are difficult to assess, including time since escape and the proportion of domestic mink in the sample. Furthermore, domestic and hybrid mink could lead to biased estimates of contamination due to differences in physiology and behavior with wild mink (Basu et al., 2009). The effects of artificial and relaxed selection experienced by domestic mink suggest that there is good reason to expect behavioral differences between domestic, hybrid, and wild mink that may lead to

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doi:10.1016/j.envres.2011.11.004

different diets of free-ranging animals, and thus, different rates of contaminant exposure (Bowman and Schulte-Hostedde, 2009; Kruska, 1996; Trapesov, 2000).

Despite the issue of escaping domestic animals, mink clearly have potential as an environmental sentinel (Basu et al., 2007). Nonetheless, increased recognition of the potential bias in studies that use mink as sentinels is required. Although some studies have taken place in regions without mink farms (e.g., Poole et al., 1998), to date, we are unaware of any study that has explicitly controlled for escaped domestic mink when assessing body burdens of contaminants in free-ranging mink samples. This is despite the large number of studies of contamination in mink (197 studies, 1981 to 2010, Web of Science, searched in July 2011 with terms “mercury and mink or PCB and mink”), and the potential for the proportion of domestic escapees in a sample to be large (e.g., 36% wild in southern Ontario; Kidd et al., 2009). A first step toward this increased recognition is to gain an understanding of how body burdens of contaminants in free-ranging domestic mink compare to those of wild mink. It would also be useful to establish some mensurative criteria that allow discrimination of domestic and wild mink in typical samples collected for environmental studies.

We compared contaminant burdens in free-ranging mink with known genetic ancestry (domestic, wild, and domestic–wild hybrids), in order to test the hypothesis that biased estimates of environmental contamination can result when genetic ancestry of mink is not considered. We predicted that free-ranging domestic and hybrid mink would have different body burdens of environmental contaminants than wild mink, and thus, that not accounting for ancestry would lead to biased inference about contamination. We assessed the extent of this potential bias in a typical study of environmental contamination in mink. As a secondary objective, we evaluated methods of excluding domestic mink from samples of free-ranging mink.

2. Materials and methods

2.1. Sample collection

The majority of mink samples used in our contaminants study were a subset of those studied by Martin et al. (2006), and details of collection methodology are provided therein. Briefly, skinned mink carcasses were collected from commercial trappers, who captured mink during winters 1998–99 through 2005–06. We assumed no temporal change in regional contamination over this sampling interval, and this assumption was supported by Martin et al. (2006) who found no significant change in PCB concentrations in mink from Lake Erie between 1979 and 1998–2003. Mink were obtained from wetlands and tributaries along the Canadian shores of Lakes Erie and St. Clair, in Ontario, Canada. Six sites were targeted for sampling, including: Eastern Lake Erie, Long Point, Western Lake Erie, Walpole Island, Lake St. Clair, and Inland (8–40 km inland from lakeshores) (Martin et al., 2006). Some additional samples collected at the same time with the same methods along the north shore of Lake Ontario were also included in our study, although they were not included by Martin et al. (2006). Trappers were provided with kits and instructions to ensure consistent methods for storing carcasses. Carcasses were wrapped in aluminum foil and stored at -20 °C until necropsy.

Necropsies were performed at the Canadian Cooperative Wildlife Health Laboratory (CCWHC), University of Guelph, Ontario under the supervision of a veterinary pathologist (B. Hunter). Prior to necropsy, carcasses were thawed, sexed, and external features examined for injuries or malformations. Mink were weighed (without pelt) and measurements of total length (from nose to tail tip) and tail length (from base to tip of tail) were recorded. Livers were excised with a hexane-washed scalpel, weighed and placed in acetone-petroleum ether washed amber glass jars and stored at -20 °C prior to chemical analysis.

2.2. Contaminants analysis

Chemical analyses of livers for organochlorine contaminants were conducted in six of eight study years at the National Wildlife Research Center (NWRC) in Hull, Québec and in two years (2002 and 2007) at the Great Lakes Institute of Environmental Research (GLIER) at the University of Windsor, Windsor, Ontario. A total of 158 samples were analyzed for sum PCBs. Samples at NWRC were analyzed according to the methods of Norstrom et al. (1988). Liver samples were thawed and homogenized and a 5-g aliquot was taken for analysis. Samples underwent neutral extraction, removal of lipids and biogenic compounds by gel permeation chromatography, followed by further cleanup using florisil column chromatography. Quantitative analysis of PCBs was performed using capillary gas chromatography, coupled with a mass selective detector (GC/MSD) operated in selected ion monitoring mode. The percent recovery of $^{13}C_{12}$-labeled internal standard of six PCB congeners ranged from 63.4% to 108.1% with a mean percent recovery of 90.7%. In the case of samples analyzed at GLIER, one standard mixture of Arochlor 1242:1254:1260 (1:1:1) was used for quantifying PCBs. The percent recovery of tribromobenzene used to measure sample recovery efficiency at GLIER ranged from 64.5% to 105.2% with a mean recovery of 83.6%. One or two quality assurance samples were analyzed with each run of mink livers. These samples consisted of 1989 diluted herring gull (*Larus argentatus*) egg pool reference material, double-crested cormorant (*Phalacrocorax auritus*) egg reference material, or carp (*Cyprinus carpio*) from the Detroit River. Samples were not adjusted for recoveries. Detection limits were 0.1 μg/kg for PCBs at NWRC and 0.03 μg/kg at GLIER. Trace concentrations were between 0.1 and 0.9 μg/kg at NWRC.

Up to and including the year 2000, samples were analyzed for total mercury using cold vapor atomic absorption spectrophotometry (CVAAS) with the Perkin-Elmer 3030B with VGA-76 according to CWS Method No. MET-CHEM-AA-03 (Neugebauer et al., 2000). These samples were thawed, freeze-dried, and digested in mineral acids prior to analysis. Samples analyzed after 2000 were freeze-dried, homogenized, and weighed out directly into nickel combustion boats. Total mercury was then analyzed using an Advanced Mercury Analyzer (AMA-254) equipped with an ASS-254 autosampler for solid samples according to CWS Method No. MET-CHEM-AA-03E (Salvato and Pirola, 1996) and then from 2006 and 2008 using CWS Method No. MET-CHEM-AA-03F.

Analytical accuracy was monitored through the use of standard reference materials, which included generally at least two of the following: DOLT-2 (dogfish liver), DORM-2 (dogfish muscle), DOLT-3, and TORT-2 (lobster hepatopancreas) from the National Research Council and Oyster Tissue 1566b from the National Institute of Standards and Technology. Mean recoveries (+SE) of standard reference materials were equal to 101.3% (+1.5%) for mercury ($n=19$ samples). Recoveries of reference materials were within the certified range for all methodologies. Analytical precision determined as percent relative standard deviation (% RSD) of replicate samples was generally below 10% for analysis of mercury. Detection limits (as dry weights) varied according to the methods used and ranged from 0.006–0.17 μg/g for total mercury.

2.3. Genetic analysis

Mink sampled for contaminant burdens were genetically profiled using the same techniques and protocols as Kidd et al. (2009). DNA was extracted from muscle tissue using a modified Qiagen protocol (Guglich et al., 1994), and amplified using primers for 10 polymorphic microsatellite loci. Details of primers and reaction conditions were described by Kidd et al. (2009). Multiplex reactions of amplified product were run on an ABI 3730 sequencer and analyzed with Genemapper 4.0 (Applied Biosystems, Valencia, CA, USA) in the Ontario Ministry of Natural Resources genetics lab at Trent University.

Only individuals that were genotyped at a minimum of 8 of 10 loci were used in subsequent analyses. Bayesian assignment tests (Structure 2.2 with the admixture model; Pritchard et al., 2000; Falush et al., 2003) were employed with $K=2$ to evaluate the probability (q) that individual mink were domestic or wild. Our previous work had shown that $K=2$ in the study area identified domestic and wild genetic clusters (Kidd et al., 2009). We included 133 mink collected for the contaminants study and successfully genotyped, as well as 223 known wild ($n=76$) or domestic ($n=147$) mink from the Kidd et al. (2009) study, which we used as outgroups to increase the accuracy of our assignment tests. Results were generated using five repetitions of 5×10^5 iterations following a burn in period of 5×10^5 iterations. Individuals were assigned to the domestic or wild clusters with a minimum membership of $q\geq0.80$, a threshold identified by Kidd et al. (2009) using a simulation analysis. Mink with a highest assignment to either cluster of $q<0.80$ were considered domestic–wild hybrids.

2.4. Data analysis

2.4.1. Contaminant burdens in domestic, wild, and hybrid mink

We initially compared sum PCBs, total mercury, and % lipids among all sampled mink pooled by genetic assignment (domestic, wild, and hybrid) across the entire set of samples. Hereafter, we refer to these 3 genetic assignments as genotypes. The 3 response variables tended to have unequal variances among the genotypes, so all comparisons were made using Kruskal–Wallis tests. We first compared sum PCBs on a wet weight basis, and mercury on a dry weight basis. *Post hoc* comparisons were made using non-parametric multiple contrast tests for unequal sample sizes (Zar, 1996).

Organochlorines bind to lipids, so if % lipids differ by genotype, this may lead to a confounding of the effects of genotype and lipids on contaminant burden. Thus, we sought to control for lipids by also comparing sum PCBs on a per lipid

basis. Again, for this comparison we pooled all samples across the study area by genotype and used a Kruskal–Wallis test.

The western basin of Lake Erie is of particular interest for organochlorine contamination, because mink from this region have been shown to exhibit large burdens of these contaminants (Martin et al., 2006; Proulx et al., 1987). Because 8 of 12 domestic mink in our study were collected in the western basin, we carried out a further comparison of PCBs from samples collected there. We first compared sum PCBs from western Erie among mink genotypes on a wet weight basis, and then on a lipid basis. We used Kruskal–Wallis tests for these comparisons.

2.4.2. Discriminating between wild and domestic mink

Given the potential of domestic mink to bias studies of environmental contamination in wild mink, we sought to evaluate methods to morphologically discriminate between domestic and wild mink, in order to exclude domestic mink from samples of free-ranging mink. To achieve this, we combined datasets from 4 different studies where mink genetic origin and morphology were known, including samples studied by Martin et al. (2006), Kidd et al. (2009), Tamlin et al. (2009), and Nituch et al. (2011). All mink from these 4 studies originated from southern or central Ontario.

There are a number of possible methods for discriminating between domestic and wild mink (Bowman and Schulte-Hostedde, 2009). Genetic methods are likely among the best, but can be time consuming and prohibitively expensive, especially if sample sizes are large. Comparing pelt quality is possible, but can be impractical, since mink are often collected for contaminants studies after being skinned by trappers. Thus, we focused on 3 morphological traits. We selected straight line snout-to-vent length (hereafter body length) as a measure of body size, to compare size without the influence of body or pelt condition. We selected condylobasal length as a measure of skull size, because it has previously been shown to discriminate well between domestic and wild mink (Tamlin et al., 2009). Finally, we compared % lipids, as measured during our contaminants study, because domestic mink were fatter than wild mink.

Mink are sexually dimorphic, so we compared domestic and wild mink within each sex, for each of the three traits. We included only mink of adult size in these analyses. Mink attain adult size within about 6 months of birth (e.g., Kruska, 1979).

We first simply compared sex-specific means for each trait, and we then conducted discriminant function analyses, with domestic mink coded as 0 and wild mink coded as 1, to estimate coefficients, classification cut-points, and classification success for each trait. For each sex, we carried out 3 bivariate analyses (one for each trait), rather than a single multivariate analysis, because the disparate nature of the sampling meant that we had insufficient samples where all 3 traits were measured on the same individual. Sample sizes varied across analyses.

3. Results

3.1. Contaminant burdens in domestic, wild, and hybrid mink

Overall, $n=133$ mink were sampled for contaminants and successfully genotyped. Of these, $n=12$ (9.0%) and $n=14$ (10.5%) had domestic and hybrid genotypes, respectively. The remaining 107 mink (80.5%), had wild genotypes.

When organochlorines from livers of these mink were compared on a wet weight basis, sum PCBs were higher in domestic than in wild mink ($\chi^2=12.27$, $n=133$, $P=0.002$), and intermediate in hybrids (Table 1). Lipids were also higher in domestic mink however, than in either hybrid or wild mink ($\chi^2=13.73$, $n=133$, $P<0.001$) (Table 1). For example, 3 of 12 domestic mink had $>15\%$ lipids, compared to 0 of 107 wild mink. Thus, we sought to control for lipid effects on organochorine estimates by comparing body burdens of these contaminants on a per lipid basis. There were no significant differences between domestic, hybrid, and wild mink for sum PCBs measured on a per lipid basis ($\chi^2=4.11$, $n=133$, $P=0.128$) (Table 2).

Total mercury measured on a dry weight basis was lower in domestic than wild mink ($\chi^2=13.14$, $n=123$, $P=0.001$). Hybrids were intermediate (Table 1). The mean (SD) mercury concentration in mink across the study area, ignoring genotype, was 3.35 (4.05) mg/kg. By controlling for genotype however, we estimated that the mean mercury concentration in wild mink in the study area was 3.79 (4.32) mg/kg. Thus, including domestic mink in the estimate could have resulted in a 13% underestimate of mink mercury burdens.

Table 1
Body burdens of contaminants and % lipids from livers of free-ranging mink (*Neovison vison*) sampled in Ontario, Canada, and genotyped to determine genetic ancestry (domestic, wild, or domestic–wild hybrid). Contaminants are the sum of 62 polychlorinated biphenyl (PCB) congeners and total mercury (Hg), expressed as mean (SD) (*n*). Concentrations are mg/kg wet weight (PCB), or dry weight (mercury). Significant differences are denoted by superscript letters. Entries in bold are for mink from the western basin of Lake Erie.

Contaminant	Domestic mink	Hybrid mink	Wild mink
Sum PCBs			
	2.21 (3.61) (12)^{A}	0.48 (1.35) (14)^{AB}	0.31 (0.79) (107)^{B}
	Range: 0.04–12.64	Range: 0.002–5.15	Range: 0.003–6.95
	3.10 (4.20) (8)	**0.35 (0.002) (2)**	**2.25 (2.21) (7)**
% Lipids			
	9.93 (13.08) (12)^{A}	2.74 (1.38) (14)^{B}	2.35 (1.40) (107)^{B}
	Range: 1.29–45.36	Range: 1.28–5.70	Range: 0.30–10.90
	13.39 (15.07) (8)	**4.00 (2.40) (2)**	**4.09 (3.36) (7)**
Hg			
	1.33 (1.79) (13)^{A}	1.90 (1.96) (11)^{AB}	3.79 (4.32) (99)^{B}
	Range: 0.18–6.61	Range: 0.26–6.09	Range: 0.11–30.00

Table 2
Body burdens of polychlorinated biphenyls (PCB) from livers of free-ranging mink (*Neovison vison*) sampled in Ontario, Canada, and genotyped to determine genetic ancestry (domestic, wild, and domestic–wild hybrid). Values are mean (SD) (*n*) concentrations in mg/kg lipid weight. Entries in bold are for mink from the western basin of Lake Erie.

	Domestic mink	Hybrid mink	Wild mink
Sum PCBs			
	77.94 (199.16) (12)	18.92 (52.72) (14)	12.39 (21.61) (107)
	Range: 0.89–706.40	Range: 0.06–201.13	Range: 0.08–144.81
	103.76 (243.91) (8)	**10.50 (6.25) (2)**	**58.44 (43.42) (7)**

We found that sum PCBs measured on a wet weight basis did not differ significantly by genotype for samples collected in the western basin of Lake Erie ($\chi^2=2.73$, $n=17$, $P=0.256$) (Table 1). Although there were no significant differences among genotypes in % lipids within western Erie ($\chi^2=2.54$, $n=17$, $P=0.282$) (Table 1), we nevertheless sought to control for lipid effects for the within-basin comparisons of organochlorines. Once again, sum PCBs measured on a per lipid basis did not differ significantly by genotype ($\chi^2=3.69$, $n=18$, $P=0.158$), although variability was high, especially in domestic mink (Table 2). Disregarding genotype of western Erie mink, the mean (SD) burden of sum PCBs on a per lipid basis was 74.13 (166.71) mg/kg ($n=17$), compared to an estimate of 58.44 (43.42) mg/kg for mink with known wild genotypes (Table 2). Thus, not accounting for genotype would result in a 27% overestimate of mean PCB contamination in wild mink from western Erie.

3.2. Discriminating between wild and domestic mink

Mean body length was 13% greater in domestic than wild male mink ($\chi^2=25.32$, $n=217$, $P<0.001$), and 15% greater in domestic than wild female mink ($\chi^2=19.66$, $n=92$, $P<0.001$) (Table 3). Domestic mink also tended to be fatter than wild mink. Lipids were significantly higher in female ($\chi^2=8.91$, $n=26$, $P=0.003$) but not in male ($\chi^2=2.07$, $n=91$, $P=0.150$) domestic mink. Mean condylobasal length was 15% greater in domestic than wild males ($\chi^2=95.57$, $n=130$, $P<0.001$), and 12% greater in domestic than wild females ($\chi^2=45.70$, $n=70$, $P<0.001$).

The discriminant function coefficient (constant) for male body length was 0.26 (-10.00), and 81% of samples were correctly classified as domestic or wild with this function. The classification

Table 3
Morphometric comparison of American mink (*Neovison vison*) sampled in Ontario, Canada. All measures were of free-ranging mink of known genetic ancestry (domestic or wild) except for condylobasal length of domestic mink, which was taken from samples of captive animals. Body length was measured as snout-to-vent length. All comparisons between genetic groups made with Kruskal–Wallis tests were significantly different ($P<0.05$), except male % lipids. Comparisons were made by sex.

	Males		Females	
	Wild	Domestic	Wild	Domestic
Body length (cm) (SD) (*n*)	38.33 (3.95) (199)	43.40 (3.69) (18)	33.56 (2.40) (79)	38.65 (3.43) (13)
Lipids (%) (SD) (*n*)	2.31 (1.19) (85)	5.52 (6.88) (6)	2.49 (2.04) (22)	19.30 (19.30) (4)
Condylobasal length (mm) (SD) (*n*)	64.33 (2.45) (65)	73.87 (2.57) (65)	58.11 (2.16) (35)	65.18 (2.65) (35)

cut-point was 40.70 cm. For female body length, the coefficient was 0.39 (− 13.26) and 88% of samples were correctly classified. The cut-point was 35.60 cm. The discriminant function coefficient for male lipid content was 0.50 (− 1.26) and 89% of samples were correctly classified. The cut-point was 3.60% lipids. The coefficient for female lipid content was 0.14 (− 0.717) and 88% of mink were correctly classified. The cut-point for female lipid content was 10.10%. Finally, the coefficient for male condylobasal length was 0.39 (− 27.51) and 97% of mink were correctly classified. The cut-point for male condylobsal length was 70.38 mm. The coefficient for female condylobasal length was 0.41 (− 25.50) and 91% were correctly classified. The cut-point was 62.80 mm.

To provide an example of an application of the discriminant models to exclude domestic mink, we reconsidered our dataset of mercury contamination in mink. Recall that mean (SD) mercury concentration in the entire mink sample, regardless of genotype was 3.35 (4.05) mg/kg ($n=123$), whereas the estimate for wild mink after excluding known domestic genotypes was 3.79 (4.32) mg/kg ($n=99$). If we did not have access to genetic data, but instead included only mink that met the body length criteria for wild individuals (males <40.7 cm, females <35.6 cm), our estimate of mean mercury contamination in wild mink would be 3.91 (4.41) mg/kg ($n=86$), close to the estimate for known wild genotypes.

4. Discussion

Our findings demonstrate the potential for domestic mink to bias studies of environmental contamination that use free-ranging mink as environmental sentinels. Contaminant burdens differed between domestic and wild mink, and these differences depended on both the type of contaminant and the spatial location of the sampled mink. When sampled across the entire Ontario study area, PCBs were higher in domestic mink than in wild mink. This appeared to be influenced in part however, by two sampling effects. First, domestic mink had more lipids than wild mink, and thus greater potential to bind organochlorines. Second, the majority of domestic mink originated from the region of the study area with the highest organochlorine contamination (the western basin of Lake Erie). Once either or both of these sampling effects were controlled by comparing contamination on a lipid basis and comparing samples originating only from western Erie, the effect was no longer significant. Even with such controls taken however, the domestic mink samples had high mean PCB concentrations and high variability. Although comparisons among genotypes were not statistically significant within the western Erie sample, we point out that the effect sizes were rather large (i.e., the lack of a significant effect was a result of the small sample size). Including domestic mink by not accounting for genotype would result in a 27% overestimate of lipid-normalized mean PCB concentrations in western Erie compared to known wild mink.

Unlike PCBs and organochlorines, mercury does not bind to lipids , and does not have a similar spatial trend in the study area (i.e., levels in western Erie were similar to other regions) (Gorman, 2007; Martin et al., 2011). Therefore, we did not use lipid weights or spatial controls in the mercury comparison. Mercury burdens were lower in domestic than in wild mink, so that not accounting for genotype would have resulted in a 15% underestimate in mean mercury burden. In other words, estimates of mercury burden that have not accounted for farm escapees may have underestimated the level of contamination in mink.

Contaminant burdens in free-ranging domestic mink may differ from burdens in wild mink due either to differences in foraging behavior or physiology, or to reduced exposure because of a lack of continuous residency in the local natural environment (Bowman and Schulte-Hostedde, 2009). Domestic mink are selectively bred for large body size and pelage characteristics, but also for tamability (Joergensen, 1985; Malmkwist and Hansen, 2002). Thus, altered behavior in domestic mink can arise from the typical tame traits that occur through domestication (Price, 1984; Trut, 1999), or from behaviors that are linked to actively selected traits (i.e., drag-along selection). For example, aggression in mink is genetically linked to pelt color (Trapesov, 2000). As such, foraging patterns in free-ranging domestic mink may be different from those of wild mink, and this could lead to different patterns of exposure to contaminants even when domestic and wild mink occur in the same local environment. Even if foraging behavior was the same however, differences in exposure to contaminants could arise. Many free-ranging mink that escape from farms will have only recently experienced natural conditions. Thus, the assumption of continuous residency of the local environment, which is required for sentinel species (Landres et al., 1988) cannot be made with confidence. Although Basu et al. (2009) state that only the most recent escapees should not reflect local contaminant levels (because steady-state conditions are achieved within 2–3 months of exposure), in our experience it can be very difficult to know, based on a physical sample, how long a given domestic mink has been exposed to the natural environment. We note that there is risk of circularity if it is assumed that a mink with a low contaminant burden has only been exposed for a short time.

We found that mercury and PCBs exhibited opposite trends. Across all geographic areas, mercury was lower in domestic than wild mink, whereas PCBs tended to be higher on average in domestic mink than wild mink. We suspect that mercury was lower in domestic mink due to reduced environmental exposure. In western Erie, many domestic mink also had lower PCB burdens

than sympatric wild mink, but mean concentrations (and SD) were high due to the influence of a small number of highly contaminated individuals. One individual in particular, had a lipid-based PCB burden of 706.4 mg/kg. This mink was an adult male who was part of a radio telemetry study in Point Pelee National Park (J. Bowman, unpubl. data). The mink was observed repeatedly during January and February 2005 foraging for Dreissenid mussels (*Dreissena* spp.) along the Lake Erie shore on the park's east beach. Mussels are poor food for mink, in part because they lead to extensive tooth damage, as they did with this individual. They are also a contaminated food source (Gewurtz et al., 2000), and may have been the source of the high PCB burden in this mink. The anecdote underscores that behavioral differences between domestic and wild mink can lead to differences in contaminant levels, as we never observed wild mink foraging on mussels during our telemetry study.

In some comparisons, hybrid mink had contaminant burdens that differed from wild mink. For example, hybrid mercury burdens were intermediate between domestic and wild mink. Since hybrid mink were born in the wild, differences in exposure between hybrid and wild mink are likely due to behavioral or physiological differences. Hybrid mink contain domestic alleles that may alter behavior, for example, by disrupting locally adapted gene complexes (Allendorf et al., 2001; Kidd et al., 2009).

Given the potential value of mink as a sentinel species (Basu et al., 2007), a main conclusion of our analyses is that it is important to try to remove samples of domestic escapees from studies that use mink as sentinels of environmental contamination. Clearly, carrying out a simultaneous analysis of mink genotypes is one approach to excluding domestic samples. For a variety of reasons, including cost and availability of expertise, this may not be a feasible approach. Based on our comparisons of 3 simple methods, we found that the highest classification success was achieved using condylobasal length of mink skulls. This success could be improved further by including a second measure, postorbital constriction (Tamlin et al., 2009). Each of the three techniques we assessed was effective however, leaving researchers with options. We note that it will likely remain difficult to exclude domestic–wild hybrid mink from contaminant studies based on morphology, since hybrids can be very similar morphologically to wild mink. Hybrid mink may bias contaminant studies due to differences in their behavior or physiology compared to wild mink.

We have provided some examples of cut-point values in morphology that could be used to identify domestic mink. In the absence of other information, these cut-point values may be useful for researchers in locations outside of our Ontario study area. We caution however, that wild mink vary in size throughout their geographic range (Stevens and Kennedy, 2005) and this variation will affect the magnitude of cut-points in local areas. We encourage researchers to assess patterns within their own data for bimodalities, which could be used to identify local cut-points.

Another conclusion from our work is that it is important to use lipid weights in studies of organochlorine contamination in mink. Livers of domestic mink have significantly higher lipid concentrations than wild mink. Due to the highly lipophylic nature of these contaminants, expressing organochlorine concentrations on a per lipid basis can control for this bias. Assessing lipids would also provide an opportunity to exclude potential domestic mink on the basis of this metric.

In summary, it appears that domestic mink can bias samples of environmental contamination in wild mink in areas where mink ranching is prevalent. We recognize however, the utility of mink as sentinel species. Thus, researchers should be aware of this potential bias, and take efforts to exclude the effects of domestic mink in studies of environmental contamination.

Acknowledgments

We received financial support for this work from an NSERC Strategic Projects supplemental grant to AIS-H and JB, the Canadian-Ontario Agreement Respecting the Great Lakes Basin, the Ontario Ministry of Natural Resources, and Environment Canada. We thank all the fur harvesters who provided samples, and Dr. B. Hunter for his assistance.

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