A short note on the effects of pollutants on the European otter (*Lutra lutra*)

D.R. Lammertsma & N.W. van den Brink 2012

Over the last decade, a successful re-introduction program resulted in the establishment of an otter population in the Wieden-Weerribben area, the Netherlands. The next stage of the reintroduction program intends to create a meta-population by facilitating the establishment of new populations at other locations as well. The current plan is to re-introduce otters in the river-system of the river Rhine and the catchment of the Meuse. These river-systems, however, are known to have elevated concentrations of certain pollutants. Therefore, information is needed on the potential risks of these pollutants for future otter populations.

This is a short note summarising the literature on risks of pollutants for otter populations. This note is not exhaustive, but will deliver some insight in their potential impact. Based on this, some recommendations will be formulated.

Population decline

In the 1960s, populations of the Eurasian otter (*Lutra lutra*) suffered serious decline in many parts of Western Europe and in some areas the species became extinct. During the 1990s the trend reversed, with many populations recovering and expanding their range (Kruuk 2006). Factors involved in the decline were habitat destruction and fractionation, isolation of populations, mortality due to hunting, traffic accidents and drowning in fish fykes. Another possible causal factor suggested was the contamination of otters with pollutants such as mercury, DDT, dieldrin and polychlorinated biphenyls (PCBs) (Mason 1989; Broekhuizen 1989; Voogt et al. 1994, Smit et al. 1998; Gutleb 2000; Christensen et al. 2010).

Hereafter, we briefly summarise the most recent toxicological studies on the impact of pollutants on otters. Most research concerning contaminants in otters focussed on the effect of PCBs and mercury.

Mercury

The heavy metal mercury can exist in several physical and chemical forms (review Gutleb 2000). Mercury in its ionic state is water-soluble and therefore bioavailable. It can be converted into organomercury compounds, which is more toxic than inorganic forms. Methylmercury (CH_3Hg) is known to accumulate in freshwater fish and is known to be a highly neurotoxic agent. Mercury induces erratic behaviour, such as falling over and moving in circles. For otters such behaviour was noted in individuals with levels in their liver exceeding 30 µg/g (Gutleb 2000).

Most data gathered on mercury levels has been based on traffic victims which could not include observations on their behaviour. Liver concentrations have been reported form Sweden (4.1-30.7 µg/g), Finland (0.05-31.0 µg/g), Orkney Islands (1.0-20.3 µg/g), Spain (3.9-17.5 µg/g), Shetlands (10-65 µg/g) and Ireland (0.15-17.0 µg/g) (Gutleb 2000; Kruuk 2006). Mercury affects individual otters, but information on the population level is lacking (Gutleb 2000; Kruuk 2006). No data is available for Dutch otters.

PHAHs

PHAHs (Polyhalogenated aromatic hydrocarbons) such as polychlorinated biphenyls (PCBs), dibenzofurans (PCDFs) and polychlorinated dibenzo-p-dioxin (PCDDs) are endocrine disrupting compounds and are reported to cause several negative effects including dermal lesions and reduced reproduction, and to be immunotoxic, carcinogenic and teratogenic (Leonards 1997; Smit et al. 1998; Gutleb 2000).. The toxicity of PCBs varies among the 209 different congeners (depending on the number and location of chlorine on the molecule) resulting in differences in tissue retention and metabolism. PCBs bio-accumulate in the food chain, with mammals, like otters, at the top of the food chain being most vulnerable.

Variation in PCB concentrations within otter population is as result of varying accumulation, but also of varying excretion and metabolism of PCBs by otters. Factors such as age, gender, body condition, reproductive status and concentration dependent metabolism are important in driving the accumulation rates of PCBs, in combination with the differential exposure by contaminated food sources. Contaminant levels in otters in Mecklenburg-Vorpommern for instance were 16 mg/kg lipid weight, with higher levels in males then in females (Griesau & Sommer 2005). Females with placental scars and active milk glands were least contaminated. Adult males (>3 years old) showed the highest levels. In otters from Denmark PCB levels ranged from 10 – 100 mg/kg lipid weight, depending on reproduction status. In this study it was also shown that males contained higher concentrations than females (Leonards et al. 1996). It was concluded that the impact of pollutants may be critical for individuals in deficiency situations (harsh winters resulting in low body condition) (Griesau & Sommer 2005)

Intermezzo: from PCB concentrations to TEQs

PCBs are a range of compounds with the same carbon skeleton, but with different chlorine substitutions (Fig. 1). Depending on the number and locations of the chlorine atoms, the PCBs differ in their toxicity. An important mode of toxicity of PCBs is similar to the toxicity of dioxins, the so-called dioxin-like toxicity. Different PCB congeners show different levels of dioxin-like toxicity, and the relative toxicity of each PCB congener can be expressed as a Toxic Equivalency Factors (TEF). When multiplying the concentration of each PCB with its respective TEF, and sum the numbers, a Toxic Equivalency Quantity (TEQ) can be calculated. This concentration is the calculated concentration in relation to dioxin-like effects.

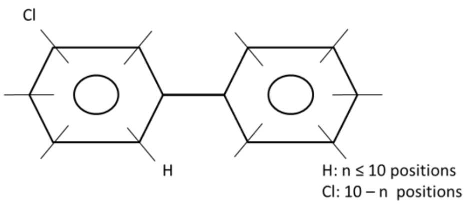

Figure 1. General structure of PCB congener.

Data from Madsen et al. (1999) indicates that an increasing concentration in PCBs is associated with an increase in the frequency of diseases (viral infections, bacterial diseases, endo parasites, pathological changes) and lower condition index in Danish otters. In a study of Murk et al. (1998) otters with an elevated PCB concentration (>2 ng TEQ/g lipid) had strongly reduced hepatic retinoid concentrations. In South-west England strong negative correlations were found between vitamin A levels and PCBs, DDT and Dieldrin in otter livers (Simpson et al. 2000). PCBs alter the metabolism of vitamin A causing developmental irregularities including fetal resorption or abortion. Vitamin A also plays an important role in animals resistance to microbial infections (Murk et al. 1998; Kruuk 2006). Roos et al. (2010) investigated the relation between observed pathological alterations in Swedish bone tissue and DDE/ PCBs. DDE is a breakdown product of DDT, an insecticide frequently applied in the past. Concentrations varied, with 1.4-970 mg ΣPCB/kg lipid weight and 0.0-24 mg DDE/kg lw in muscle tissue. Positive relationships were found for ΣPCB and cortical bone variables (area, thickness, content), while no significant relationship was found with DDE. Van den Brink & Jansman detected variation in PCB patterns in otters from the reintroduction program in The Netherlands, indicative of induced liver enzymes by PHAHs (Van den Brink & Jansman 2006).

Toxic effect levels

Tissues

The proposed tissue-based effect level of 50 mg PCB/kg lipid weight for otters was based on an extrapolation of toxicity tests on American mink (*Mustela vison*) causing reproductive failure (Jensen et al. 1977). In a recent long term study, mink already suffered reproductive toxicity at 12 mg PCB/kg lipid weight (Brunström et al. 2001). Since it is very likely that there are differences between mustelid species regarding their sensitivity to PCBs, it is debatable whether these extrapolations within the group of mustelids are allowed (Leonards 1997; Kruuk 2006).

Based on a study on environmentally exposed feral and captive otters, the no effect level for vitamin A reduction by PCBs appeared to be 2-5 ng TEQ/g lipid weight, measured either in blood plasma or liver (Murk et al. 1998). In the Shetlands during the 1990s, PCB values amounted to a mean of 210 mg/kg lipid weight in the liver (Kruuk 2006). This included lactating (reproducing) females with values up to 20 times higher than the concentration causing reproductive failure in mink. Since not all reproductively active females were reproducing, it was concluded that there was a fair chance that individuals were affected (Smit et al. 1998). PCB levels in liver and fat tissues of reintroduced otters in the Netherlands averaged 38mg/kg lipid weight (0.01-362mg/kg). None of the autopsies on otters (N=99) found dead during the past ten years after the start of the reintroduction in 2002, did reveal clinical abnormalities. Reproduction seemed normal; the number of young amounted to 1,7 ± 4,9 (Koelewijn et al. 2010), which is normal compared to other European populations (1.5-1.7 pre-dispersal litter size; Ruiz-Olmo et al., 2005; Sulkava, 2006). In conclusion, there are no data available showing the toxic effect levels of PCBs for the Eurasian otter on the population level. Circumstantial evidence makes it likely that the otter is less sensitive to PCBs compared to the American mink for which the toxic effect level was estimated at 50 mg/kg lipid weight.

Spraints

Spraints are excreted by otters, and may be used for monitoring of concentrations of contaminants in otters. Based on a study on otters from the reintroduced population in the Netherlands, threshold levels of approximately 1-2.5 mg/kg (lipid weight) were proposed for spraints reflecting PCB patterns of otters, i.e. lacking metabolisable PCB congeners (Van den Brink & Jansman 2006). Concentrations in spraints varied from 0.5-2.3 mg/kg, while spraints with fish remains contained concentrations up to 6.4 mg/kg. The concentrations with the otter signatures were in the range of the threshold levels. In a study on otter spraints in Wales, a no effect level of 4 mg/kg was proposed (Mason & MacDonald, 1994). However, this was based on all types of spraints, including ones that are dominated by fish remains which do not reflect internal concentrations of the otter that produced the spraint. This was also shown in another study, in which PCB concentrations in spraints which were not specifically selected to reflect internal body concentrations, indeed did not show a significant relationship with tissue concentrations in otters (Mason and Macdonald, 1994). Hence, threshold levels based on such data should be used with care. Nevertheless, it was concluded in both studies that in specific regions of South West England PCBs may affect the local otter populations (Mason & Macdonald 1993, 1994).

Effect at the population level

In many areas in Europe where populations declined or went extinct, high concentrations of PCBs have been found. In general average PCB levels of 50-180 mg/kg lipid weight corresponded with declining populations and mean levels <30 mg/kg lipid weight with thriving populations (Smit et al. 1998). This suggests a correlation between population development and PCB levels (Gutleb 2000; Roos et al. 2001). The exception to the rule is a flourishing population on Shetland with high mean PCB levels (Kruuk 2006). In Denmark the population was stable or in some regions expanding and it did not appear that PCB-induced diseases at the level of the individual had a relevant detrimental effect on the population level (Gutleb 2000).

The lack of evidence of effects of PCBs at the population level, although individuals may be affected, may be explained by ecological interactions. For instance, in the Dutch reintroduction program it was clear that reproduction depended on only a small proportion of all males. Turnover rate of the population was high due to high mortality of adult males. Especially in situations with a low turnover rate of the population PCB accumulation may reach critical levels. Reproduction can be hampered when the dominant male, responsible for most reproduction, becomes unsuccessful. If other males that could replace him are affected by PCBs as well reproduction of the total population may decline. This effect becomes only apparent in case of multi-stress situations. In such a scenario, the exposure to PCBs may not be problematic at population level, but it should be noted that in case of additional stress, effects may be much more pronounced in populations under PCB exposure. The population as a whole may act on an ecological smaller base under PCB exposure.

Ecotoxicological suitability of the Rhine and Meuse river-system in the Netherlands as habitat for otters

Generally, the water quality in the Netherlands, including that of the large rivers, has increased over the last decades. PCB levels in eel during the 1980s ranged in the reintroduction area from 272-414 Σ7PCB µg/ kg lipid weight (Werkgroep Otter en PCB-verontreiniging 1990). In the river Regge and lower Dinkel levels were 1150-1200 µg/ kg lipid weight. Highest levels were found in the river Vecht and the Zwartewater (1400-3647 µg/ kg lipid weight). The PCB concentrations in the Dutch river-system, including the Rhine and IJssel, exceeded in 2010 the threshold value imposed by the Water Framework Directive (Directive 2000/60/EC) by 1,2 - 5 times the standard of 8µg/kg dry weight particulate matter **(**Snijders 2011). Over the past years, there is generally a slightly decreasing trend. Inland waters, including the Oude IIssel, currently meet criteria for pollution (Waterschap Rijn en IJssel 2012). PCB levels in eel from Lobith, where the river Rhine enters the Netherlands, show a steady decline in time. However, levels in eels more downstream and in the Meuse river show steady concentrations since 1990 (de Boer et al. 2010; Kotterman & van der Lee 2011). Variance in PCB levels in eel is high and is influenced by length, sex and habitat heterogeneity (Kotterman & van der Lee 2011). Larger eels have higher TEQ values. TEQ values therefore of individual eels may exceed thresholds for human consumption even if PCB values on the population level do not surpass the threshold value. Thresholds in the Meuse and Rhine for human consumption are exceeded. In eels from the river Meuse and the Waal the concentrations in eel are relatively high, approximately 6.2 to 8.5 mg/kg (7∑PCB, lipid weight). This corresponds to a PCB uptake by otter of approximately 0.98 mg PCBs/day (assuming 20% fat in eel (de Boer et al. 2010 and 650 g daily fish intake by otter). This corresponds with approximately 250-300 pg TEQ/day (de Boer et al. 2010). Leonards (1997) derived a No Adverse Effect Dietary Concentration (NOAED) of 500 pg TEQ/day, which is slightly higher than otters would accumulate, eating solely eel from the larger rivers. However, it should be noted that 7∑PCB lacks the most toxic congeners, which are not included in the calculation of the daily intake. Research on Polecats (Leonards et al. 1994) showed that the contribution of for instance PCB126 in the derivation of the TEQs is >75%. However, these more toxic congeners are included in the derivation of the threshold by Leonards et al. (1997), and when subtracting the contribution of just PCB126 this threshold could be lowered to 100-150 pg/day (remaining TEQ is approximately 25% of 500 pg/day). When applied to the current situation, it appears that in case otters would forage solely on eel, thresholds of toxicity are likely to be exceeded. This is, however, a not very realistic assumption. During the 1980s ca. 10% of the otter diet consisted of eel (Bekker 1988 cited in Werkgroep Otter en PCB-verontreiniging 1990). Eel densities nowadays are low, and generally no eel remains were found in otter spraints collected in the otter core area of De Wieden/Weerribben (pers communication Freek Niewold; Niewold 2012). Moreover, otters dwelling in river systems will not only forage in the main river stream, but also (and probably mainly) in adjacent waters which have a better water quality with less contaminated fish (Kotterman & van der Lee 2011). It is therefore likely that otters will forage on other, most likely less contaminated prey items. In this light it remains uncertain to which levels otters will be exposed. Nevertheless, it is likely that in worst case threshold concentrations for effects at the individual level may be reached.

Intermezzo

For the potential significance of risks that PCBs may pose to otters in the large rivers in the Netherlands it may be useful to address other studies on fish-eating species. A major study was conducted on cormorants (*Phalacrocorax carbo sinensis*) in the Biesbosch area. In this area sedimentation of material carried by the river is an important process which results in relatively high levels of contaminants in this area. In the late 1980s it was assessed why the breeding success of cormorants in this area was very poor (Boudewijn and Dirkse, 1995). In that study it was concluded that exposure to organochlorine pollutants was the most likely cause of the observed poor reproductive success. Exposure to DDTs resulted in egg-shell thinning and exposure to PCBs in embryo-toxicity. Later, the breeding success increased again, and exposure to contaminants seemed to have decreased. This, however, was not related to decreasing environmental levels of organochlorines, but to a shift in diet (Boudewijn et al. 1994). It appeared that cormorants were foraging on smaller, younger fish which were less contaminated, and therefore the exposure decreased.

Sensitivity and vulnerability of otter populations to exposure to pollutants

A substantial body of research has been devoted to the sensitivity of otters to exposure to different types of organochlorine pollutants. Much of this is based on research on mink. Other research on the sensitivity of otter to contaminants is based on sub-individual effects, for instance on vitamin A (Murk et al. 1998). However, no studies are available that describe dose-response relationships between population dynamics of free-roaming otters and organochlorines. When applying the threshold levels derived from mink studies, or from physiological endpoints, PCB levels in thriving otter populations exceed these thresholds by far, see for instance otter populations from the Shetlands (Kruuk 2006). This may be caused by the fact that the Eurasian otter is less sensitive compared to mink, or by ecological (feedback) processes that neutralise effects at the individual level, masking effects at the population level (see example above). If otters are less sensitive than mink, the risks organochlorines pose to otter populations may be overestimated. In case effects are masked by ecological interactions, risks may appear to be overestimated, but the ecological width that an exposed otter population acts on may be smaller compared to a non-exposed situation. The resilience of otter populations to deal with other stressors may be smaller under exposed conditions, indicating that an exposed population may be much less capable to deal with additional stressors.

Conclusion

The serious decline of European otter populations since the 1960s coincided with increasing PCB and DDE/DDT levels in the environment, but these observations were based on correlative studies. The recovery of populations in Europe during the 1990s coincided the other way round. Toxicological thresholds for PCBs are based on mink studies. However, these thresholds may not directly be applicable to effects on populations as evidence has shown that populations with high PCB concentrations in fat tissue appeared to be healthy. Observations on PCBs in otters from the re-introduction program, tissues and spraints, showed that PCB concentrations were in the same range as threshold levels of effects at individual level, although the population appeared to be thriving. So, risks on effects at the individual cannot directly be translated into risks at the population level. Nevertheless, it is not clear whether an exposed population, showing no indications of direct effects, will be sustainable in combination with other stressors. PCB-levels currently detected in eel from the large rivers in the Netherlands appear to be high enough to affect individual otters, but it remains questionable whether effects will become noticeable at the individual or population level. Otters dwelling in river systems will not only forage in the main river stream, but also (and probably mainly) in adjacent waters which have a better water quality with less contaminated fish. Habitat use and diet of otters in Dutch river systems has not been studied. However, this may not lead to the conclusion that pollution is irrelevant in respect to otter population dynamics. Contaminants may affect the resilience of the total population towards other stressors, and in a multi-stress situation effects may become more pronounced. It is therefore essential that in the re-introduction program, contaminant levels are monitored in individual otters in concordance with demographic variables related to the population dynamics. This will shed a light on the potential effects of pollutants on the population dynamics of the otter.

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