CHEM Trust

Protecting humans and wildlife from harmful chemicals

PERSISTENT ORGANIC POLLUTANTS AND INDICATORS OF OTTER HEALTH: OTHER FACTORS AT PLAY?

A CHEM Trust report by Dr Eleanor Kean, Gwynne Lyons and Dr Elizabeth Chadwick

February 2013

This is a report commissioned by CHEM Trust and undertaken by Cardiff University.

The Cardiff University Otter Project (CUOP) has received funding from the Environment Agency (EA) since 1992 to conduct post-mortem examinations of otters found dead in England and Wales. Numbers found each year have steadily increased and CUOP have, to date, examined over 2000 otters from all over the UK, predominantly killed in road traffic accidents.

The original focus of the project was to use tissue samples for contaminant analysis, and the use of otters as sentinels for contaminants remains a key strength. In addition, CUOP now uses the archive of specimens and data to conduct a wide range of research, monitoring health and disease and informing conservation. Much of this work is collaborative with a wide range of other universities and organisations, and includes research into landscape genetics, chemical communication, parasitology, diet, and population structure.

For further details see www.otterproject.cf.ac.uk

This report was commissioned by CHEM Trust, a UK charity working to protect humans and wildlife from harmful chemicals. CHEM Trust's particular concerns relate to chemicals with hormone disrupting properties, persistent chemicals that accumulate in organisms, the cocktail effect and the detrimental role of chemical exposures during development in the womb and in early life. CHEM Trust strongly supports the conservation of biodiversity and believes in the importance of wildlife protection. Furthermore, monitoring wildlife populations can provide vital insights into contaminant related threats to human health.

Both wildlife and humans are at risk from pollutants in the environment. CHEM Trust's vision is a chemicals industry that plays no part in causing impaired reproduction, deformities, disease, deficits in brain function, or other adverse health effects. Human exposure to some undesirable chemicals may arise from contamination of the food chain and from the use and disposal of many everyday products such as TVs, computers, cars, construction materials, toys, toiletries and cosmetics.

CHEM Trust is committed to engaging with all parties, including regulatory authorities, the chemical industry, scientists and medical professionals to increase informed dialogue on the harmful role of some chemicals. By so doing, CHEM Trust aims to secure agreement on the need for better controls over certain chemicals, and thereby to prevent disease and protect both humans and wildlife.

For further details please contact Gwynne.lyons@chemtrust.org.uk
For other CHEM Trust publications see the back of this report and www.chemtrust.org.uk

About the authors

Dr Elizabeth Chadwick is an Independent Research Fellow at Cardiff University and Manager of the Otter Project since 2004. Under her leadership the Project has expanded, and now includes an active team of researchers. Her research not only furthers our understanding of this elusive European protected species, but also uses it as a model organism to investigate key ecological principles and processes.

Dr Eleanor Kean is a Research Associate at Cardiff University. She worked for several years in wildlife conservation before completing her PhD research on odour communication in otters. She is active in science policy having previously gained a competitive Parliamentary Fellowship from the British Ecological Society. Her current research at Cardiff University focuses on ecotoxicology with the Otter Project, and the transmission and detection of wildlife disease.

Gwynne Lyons is Director of CHEM Trust and previously worked for WWF-UK, before setting up CHEM Trust in 2007 with co-director Elizabeth Salter-Green. She has been a member of the Health and Safety Commission's Advisory Committee on Toxic Chemicals, and was a member of the UK Government's Advisory Committee on Hazardous Chemicals from 2001-2008. In 2008, Gwynne featured in The Independent on Sunday list of Britain's top 100 environmentalists as "Britain's most effective expert on toxic chemicals".

This report should be cited as: Kean EF, Lyons G, & Chadwick EA (2013) Persistent organic pollutants and indicators of otter health. A CHEM Trust report.

Further copies of this report can be downloaded from www.chemtrust.org.uk or www.otterproject.cf.ac.uk

Acknowledgements

CHEM Trust gratefully acknowledges the support of the Esmée Fairbairn Foundation.

The authors would also like to thank the many individuals who have collected and transported otter carcasses to Cardiff University. Thank you also to Otter Project Research Assistants past and present and the many volunteers who have assisted with post mortem examinations.

Contents

Abbreviations

CUOP	Cardiff University Otter Project
DDE	dichlorodiphenyldichloroethylene
DDT	dichlorodiphenyltrichloroethane
EA	Environment Agency
GLM	generalised linear model
HCBenz	hexachlorobenzene
HCH	hexachlorocyclohexane (a, b, g and d isomers are indicated as a-HCH, etc).
OCs	organochlorine pesticides
op	ortho para (e.g. ortho para isomer of DDE)
pp	para para (e.g. para para isomer of DDE)
PCB	polychlorinated biphenyl
POP	Persistent Organic Pollutant
RTA	road traffic accident
Sum PCB TEQ	the sum TEQ of PCB congeners 105, 118 and 156
TDE	dichloro-bis(p-chlororphenyl)ethane
TEQ	toxic equivalence
WHO	World Health Organisation
WILDCOMS	Wildlife Disease and Contaminant Monitoring and Surveillance network

Summary

- The Eurasian otter *Lutra lutra* is an indigenous top predator of aquatic systems in the UK. Levels of pollutants in otter tissues can be used as an indicator of pollutants in the aquatic environment.
- It is generally accepted that bioaccumulation of certain persistent organic pollutants (POPs) caused a crash in otter populations in the UK in the 1970s. Since then, levels of these POPs have gradually declined and otter populations have increased.
- This report shows that despite the population increase, there are indicators which suggest that otters may not be in optimal reproductive health.
- The current report examines whether otter health (as indicated by body size, reproductive indicators, organ weights, parasites and abnormalities, measured post mortem) has improved as concentrations of some POPs frequently detected in otter livers (Dieldrin, DDT, PCBs and HCBenz) have decreased.
- The majority of health indicators examined (11/17) show some association with concentrations of at least one of the measured POPs, but trends vary between pollutants and between subgroups of the otter population. Thus, the POPs analysed in the current report do not appear to show any consistent negative associations with the selected health indicators. This therefore suggests that other factors, potentially including newer pollutants that are not currently tested for, are driving the observed changes.
- Several indicators of male reproductive health give cause for concern for the health of otters, including a decrease in baculum weight over time, and an increase in reproductive abnormalities including cysts on the vas deferens and cryptorchidism. Since the last otter in this report was found (2009), over 600 more otter carcasses have been examined and cysts on the vas deferens are now much more common, occurring in 11% of adult male otters examined. Continued monitoring of reproductive indicators should receive high priority.
- It is recommended that monitoring of pollutants and health in top predators such as the otter should be expanded. Given the potential role of endocrine disrupting chemicals in reproductive health, particular attention should be paid to these chemicals, and to reproductive health indicators in such species. Additional, and more sensitive indicators should be sought, to give a broader picture of health of the otter - one of the UK's most popular species and an indicator of a healthy freshwater environment.
- Failure to regularly revise the suite of pollutants measured may lead to a false sense of security and cause emerging threats to be missed. There is a need for an effective early warning system in order to protect the otter and the freshwater environment.

1. Introduction

1.1 Background

The Eurasian otter *Lutra lutra* is a popular species, highly valued by the general public. In addition, it is a top predator of aquatic systems in the UK and levels of pollutants in otter tissues can therefore be used as an indicator of pollutant levels in those systems. The well documented decline of otters in the UK and Europe in the last century has been linked to bioaccumulation of persistent organic pollutants (POPs) including organochlorine pesticides (OCs) and polychlorinated biphenyls (PCBs), although other factors such as food availability and habitat quality are also likely to have contributed (reviewed by Chanin, 2003). These POPs, for example dieldrin and DDT, have been banned in the UK for several decades and are now included in a United Nations treaty (the Stockholm Convention on Persistent Organic Pollutants, 2001) that aims to eliminate or restrict their use globally. At their lowest point in the late 1970s, otters were absent from parts of Scotland, much of Wales and most of England. Indeed, national surveys in the late 1970's found evidence of otters at only 6% of sites examined in England (Lenton et al., 1980) and 20% of sites examined in Wales (Crawford et al., 1979). Otter populations in the UK have since increased: the latest national surveys found otter signs in 58.8% of sites in England (Crawford, 2010) and 89.9% of sites in Wales (Strachan, 2010). Since the recent national survey of England was published, signs of otters have been found in Kent, which shows that otters are now once again in every county in England.

The Otter Project at Cardiff University, with funding from the Environment Agency, has been collecting otter carcasses for post mortem examination since 1992. Initially collection was from Wales and mid, east and north England, but since 2007, from all of England and Wales. Details of the otter collection scheme and post mortem examination methods are available at www.otterproject.cf.ac.uk. The data and tissues collected are used for a range of research. Analysis of otter tissues from England and Wales has clearly shown that some organic pollutants persist in aquatic ecosystems decades after they were banned (Simpson 1998; Bradshaw and Slater 2002; Simpson 2007; Chadwick 2007). Such POPs have been shown to be associated with some physiological and developmental defects (Simpson 2007).

Between 1992 and 2009 Cardiff University and the Environment Agency monitored 38 POPs in otter livers, these were; DDT (6 isomers and derivatives); PCBs (23 congeners); HCH (4 isomers of hexachlorocyclohexane); dieldrin (4 isomers and derivatives); HCBenz (hexachlorobenzene) (listed in Appendix 1). Cardiff University Otter Project recently reported to the Environment Agency on the long term (1992 to 2009) and widespread (England and Wales) decline in nine of the thirteen most frequently detected of those POPs (Kean and Chadwick, 2012). CHEM Trust subsequently commissioned Cardiff University to investigate whether declines in the POPs measured between 1992 and 2009 were associated with improved otter health, as determined by the evaluation of certain indicators recorded during post mortem examination. The 13 most frequently detected substances from the 38 monitored became a focus of this report. The rationale for this was to see if otter health was improving in tandem with the reported declines in the levels of these 13 frequently detected POPs.

If otter health has not improved in recent years as these POPs have declined, this would suggest that other factors are impacting otter health. This might include other chemicals that are now being used, but which are not currently monitored in otters. Studies in other organisms show increasing exposure to some 'new' POPs, for example polyfluoroalkyl substances (Houde et al., 2006). There is also mounting concern about hormone disrupting chemicals in the environment, as these can be detrimental to health, even though they may not be as persistent and bioaccumulative as POPs (Kortenkamp, 2007).

The past declines in otter populations in the UK, their long recovery time and the continued fragility of recovery in some areas (e.g. Kent) raise concerns about potential declines in the future. As a top predator, otters are clearly vulnerable to food chain contamination and freshwater pollution, and analyses such as those in this report are vital both for early detection of negative impacts on otter health and for assessing the health of the aquatic environment. Current analysis of otter tissues by the Environment Agency is limited to some already banned POPs (listed in Appendix 1): the levels of numerous other chemicals currently in use are not monitored in otters, and so it is not possible to directly assess their potential impacts.

1.2 Objectives

Analyse potential indicators of otter health;

- Size and condition
- Organ weights
- Parasite burden
- Reproductive indicators
- Abnormalities and fighting injuries

to assess whether;

i) otter health has changed over 18 years (1992 to 2009)
ii) health indicators correlate with levels of 13 frequently occurring POPs.

2. Methods

2.1 Sample collection and post mortem examination

Otter carcasses are collected by members of the public and organisations including the Environment Agency, Countryside Council for Wales, UK Wildlife Trusts, the police and local authorities. Information recorded with each carcass includes the date and location of death. Carcasses are submitted to Cardiff University frozen, and stored at -20°C prior to performance of a standard post mortem protocol (see www.otterproject.cf.ac.uk). Liver samples are retained (except where severely damaged or decomposed), wrapped in aluminium foil and stored at -20°C prior to laboratory analysis for pollutants.

Table 1. Count of samples analysed

	Sex		
Age	Female	Male	Total
Adult	154	280	434
Sub-adult	138	143	281
Juvenile	17	23	40
Total	309	446	755

Here we report on 755 otters from which livers were retained (Table 1) collected between 1992 and 2009 (from across all Environment Agency Regions: Anglian, Midlands, North East, North West, Wales, South West, Southern and Thames [Regional boundaries as defined in April 2010]) (Figure 1).

Additional variables determined at post mortem and used in the current analysis include sex, age class, cause of death and health indicators (Table 2). Data for some individuals are incomplete, due, for example, to severe damage as a result of road traffic accident (RTA).

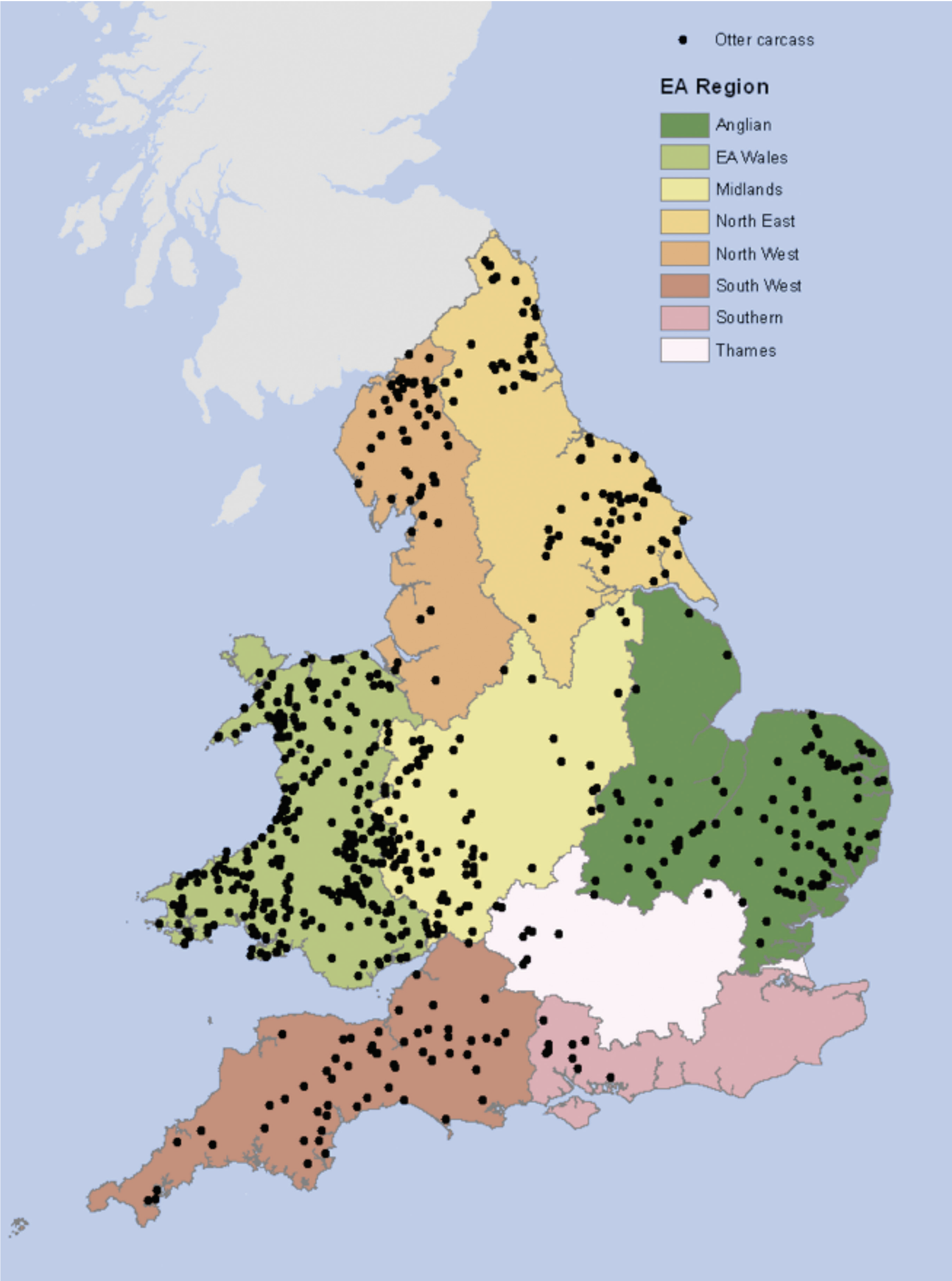


Figure 1. Distribution of samples analysed

34 individuals are not shown because National Grid References were not provided. Environment Agency Regional boundaries (as used in statistical models) are indicated.

Table 2. Data collected from each otter

		Definition	**Use in statistical models** d=dependent variable, i=independent variable
Basic data			
Date found	Year	1992-2009	i
	Season	Winter= December-February, Spring= March-May, Summer=June-August, Autumn=September-November	i
Location	Region	Environment Agency Regions: Anglian, Wales, Midlands, Southern, Thames, South West, North-East, North West, as defined by the EA in April 2010.	i
Otter data	Sex	Male or female	i
	Age class	Categorised as juvenile (females <2.1kg, males <3kg), sub-adult (females ≥ 2.1kg with no sign of reproductive activity, males ≥ 3kg with a baculum <60mm in length) or adult (females with signs of reproductive activity, males with baculum ≥ 60mm).	i
Carcass data	Cause of death	Categorised as RTA, assumed RTA (older records with limited details), drowned, fighting, shot/snared, infection/emaciation, infection/emaciation including bites, unknown.	i
	Carcass condition	Categorised as fresh, slightly or moderately decomposed (seriously decomposed individuals were excluded from analysis)	i
Potential health indicators			
Size and condition	Body length	Measured from nose to tail-tip to the nearest 5mm.	both
	Condition	Calculated based on Kruuk and Conroy (1991), separate calculations for male and females to allow for sexual dimorphism. $W/5.02L^{2.33}$ for females $W/5.87L^{2.39}$ for males W=weight (kg), L=length (m)	both
Reproductive indicators	Baculum length	Measured in mm, to the nearest mm	d
	Baculum weight	Measured in g	d
	Testes weight	Left and right weighed separately in g and summed	d
	Recent female reproduction	Presence/absence of signs of recent reproduction (pregnancy or lactation) in adult females.	d
Parasite burden	Gall bladder parasites	Presence/absence of trematode parasites *Pseudamphistomum truncatum* or *Metorchis albidus* in the gall bladder	d
	Toxoplasma	Presence/absence of antibodies against T*oxoplasma gondii* in the blood	d
	Ectoparasites	Presence/absence of *Ixodes* ticks	d
Organ weights	Adrenal	Measured in g; sum of left and right	d
	Kidney	Measured in g; sum of left and right	d
	Spleen	Measured in g	d
	Heart	Measured in g	d
	Liver	Measured in g	d
Abnormalities	Kidney stones	Presence/absence	d
	Fighting injuries	Presence/absence	d
	Infection	Presence/absence	NA
	Cryptorchidism	Failure of one or both testes to descend through the abdominal wall via the inguinal canal	NA
	Cysts and swellings	Presence/absence	NA

2.2 Laboratory analysis of POPs

All pollutants were measured in µg kg-1 wet weight by the UK Environment Agency's National Laboratory Service. All Environment Agency National Laboratory Service laboratories are accredited to ISO 17025 by United Kingdom Accreditation Scheme. Full details of analytical methods can be accessed on the Cardiff University Otter Project website, www.otterproject.cf.ac.uk.

2.3 Data analysis

Previous analysis (Kean and Chadwick, 2012) found that of the 38 pollutants measured in otter livers between 1992 and 2009, thirteen were frequently detected (concentrations were >detection limit in 80-100% of samples). This report focuses on these 13 pollutants: dieldrin, para para isomers of DDE and TDE, hexachlorobenzene and PCB congeners 105, 118, 128, 138, 153, 156, 170, 180 and 187.

Five classes of health indicators (size and condition, reproductive indicators, parasite burden, organ weights, and abnormalities, Table 2) formed the dependent variables of 17 statistical models.

2.3.1 Preliminary data treatment

Preliminary treatment of pollutant data is described elsewhere (Kean and Chadwick, 2012). In brief, raw data were screened for inconsistencies or missing values prior to analysis and removed from the dataset where appropriate, resulting in the final dataset of 755 otters. For statistical purposes, non-detected concentrations of pollutants were assigned a value of half the limit of detection. For the current report, the toxic equivalence factor (assigned by WHO) of dioxin-like PCB congeners (105,118 and 156) were used to calculate the toxic equivalence (TEQ). Where all three congeners were measured, the sum of these three TEQs (Sum TEQ) was used in statistical analyses rather than the individual measured levels of these three PCBs.

2.3.2 Statistical analyses

Where data were sufficient, generalised linear models (GLMs) were employed to analyse each health indicator separately as a dependent variable using R (version 2.13.1; R Development Core Team, 2011). GLMs allow for unbalanced sampling (due to the necessarily ad hoc nature of otter collection, data derived are unbalanced, that is the representation of different Regions, sexes, age-classes etc varies considerably between years) while evaluating the effects of multiple predictor variables on health indicators. Full details are given in Appendix 2.

3. Results

3.1 Recorded levels of pollutants and summary of health indicators

Levels measured ranged from 0.5 to 7660 µg kg^{-1}(wet weight); the highest value was of pp DDE (Table 3). Median values ranged from 6 to 129 µg kg^{-1}. Mean values tend to be skewed by a small number of high values, therefore median is a better measure of average pollutant levels.

Table 3. Average and range of pollutant levels in µg kg^{-1} of liver tissue

	n	Min.	Max.	Median
Dieldrin	741	0.50	1710	83.55
ppDDE	742	0.50	7660	129.00
ppTDE	603	0.50	430	10.60
PCB128	584	0.50	229	7.52
PCB138	724	0.50	2530	45.80
PCB153	711	0.50	1830	60.85
PCB170	615	0.50	520	19.90
PCB180	732	0.50	1410	41.60
PCB187	627	1.12	1269	36.20
HCBenz	661	0.50	479	12.30
Sum PCB TEQ	639	0.0001	0.0549	0.0010

N.B not all livers were tested for all pollutants

The average, minimum and maximum value for each otter health indicator is detailed in Table 4. Kruuk and Conroy's (1991) condition index suggests that a condition value of 1 is 'normal'; values less than 1 indicate lower than typical weight for a given body length, and vice versa. All health indicators vary widely, for example with the sex and age class of the individual otter.

Table 4. Average and range of otter health indicators

Health indicator	n	Min.	Mean	Max.
Condition	697	0.47	1.02	1.71
Body length (mm)	708	425	1036	1308
Baculum length (mm)	409	17.00	57.94	80.00
Baculum weight (g)	282	0.10	3.77	9.80
Testes weight (g)	243	0.22	5.97	15.08
Recent female reprod (yes/no)	152		37% positive	
Gall bladder parasites (yes/no)	313		18% positive	
Toxoplasma gondii (yes/no)	204		42% positive	
Ectoparasites (yes/no)	377		44% positive	
Adrenals (g)	485	0.07	0.80	2.65
Kidneys (g)	454	7.58	45.99	114.10
Spleen (g)	328	0.62	27.03	70.93
Heart (g)	253	4.80	50.89	117.00
Liver (g)	277	22.53	200.06	452.61
Kidney stones (yes/no)	673		15% positive	
Fighting injuries (yes/no)	668		16% positive	

3.2 Temporal trends in health indicators

Three of the health indicators analysed had a significant association with the year in which the otter was found dead: baculum weight, adrenal weight and fighting injuries. There has been a small but significant decline in baculum and adrenal weights between 1992 and 2009 and in the same time period the incidence rate of fighting injuries has increased (Figure 2). Testes weight and all parasites have only been measured consistently since 2004 and so examination of time trends in these health indicators was limited.

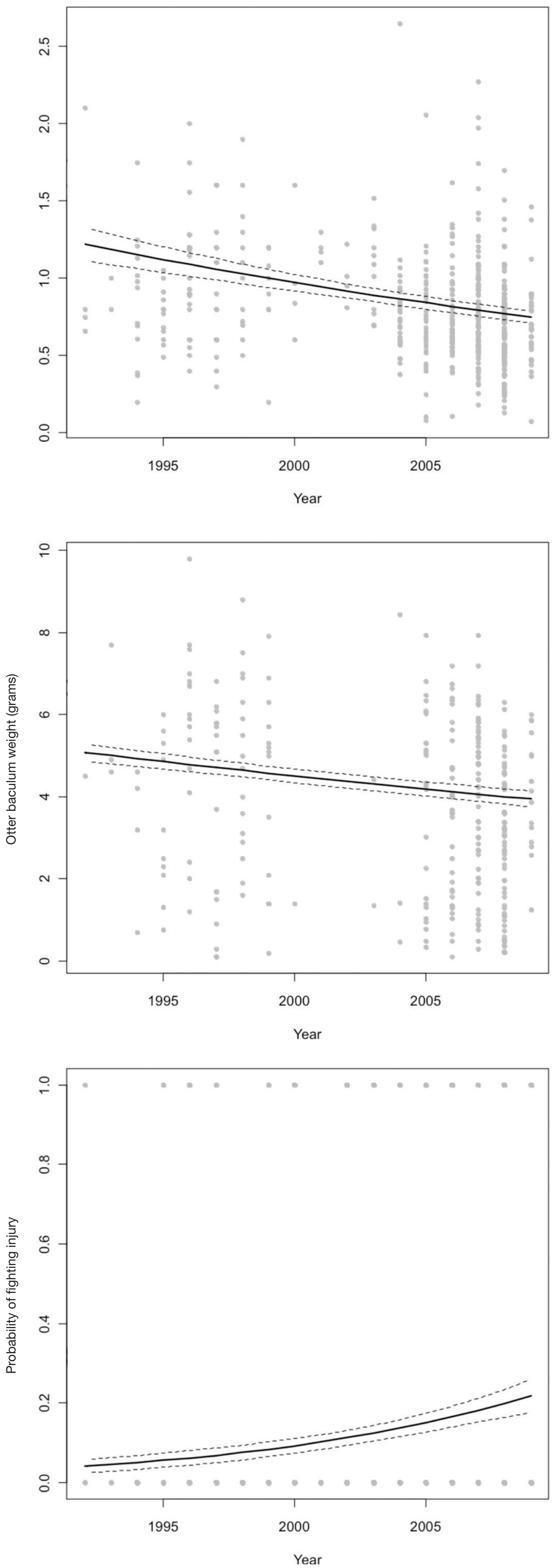


Figure 2. Temporal trends in health indicators. Dashed lines indicate +/- 1 standard error. Raw data, plotted in the background in grey, covers the whole dataset, whereas model results are predictions where all other variables (e.g. body size) are controlled as specified in Appendix 2.

3.3 Pollutants associated with health indicators

Significant associations with pollutants varied considerably between health indicators (see Table 5 for a summary and Appendix 3 for full details). All pollutants were significantly associated with at least one health indicator, but while some showed multiple associations within a category (e.g. dieldrin showed a significant association with three of five organ weight indicators, but no association within any other category of health indicator), others showed single associations across multiple categories (e.g. PCB 128 showed an association with a single health indicator in each of four categories). PCB 153 and PCB138 had the most numerous significant individual associations (six and five respectively), while PCB 128 had associations across most categories (four). In many cases, the nature of the significant relationship showed an interaction with other variables, for example with sex (e.g. suggesting a positive correlation between pollutants and health indicators for males but a negative correlation for females). The adjusted R-squared (or pseudo R-squared for binomial models) was very low for some models, indicating that the dependent variables (pollutants, otter age, sex, location etc) do not explain the variation in the health indicator very well. This suggests that other factors, not included in the models presented, contribute to the measured variation in health indicators.

Table 5. Summary of pollutants that explained variation in otter health indicators. Significant associations are indicated with a ✓. Numbers in parentheses indicate the number of health indicators for which a relationship was statistically significant / the total number of potential health indicators in the given category.

Pollutants	Number of health indicators	Type of health indicators				
		Size	Reproduction	Parasites	Organ weights	Abnormalities
Dieldrin	3				✓ (3/5)	
ppDDE	2		✓ (1/4)		✓ (1/5)	
ppTDE	2	✓ (1/2)			✓ (1/5)	
PCB128	4	✓ (1/2)	✓ (1/4)	✓ (1/2)	✓ (1/5)	
PCB138	5	✓ (2/2)	✓ (2/4)	✓ (1/2)		
PCB153	6	✓ (1/2)	✓ (1/4)	✓ (2/2)		✓ (1/2)
PCB170	2	✓ (1/2)			✓ (1/5)	
PCB180	2	✓ (1/2)			✓ (1/5)	
PCB187	4	✓ (1/2)	✓ (1/4)		✓ (2/5)	
HCBenz	1				✓ (1/5)	
Sum PCB TEQ	2	✓ (1/2)	✓(1/4)			

3.4 Size and condition

3.4.1 Condition

An index for condition was calculated for 697 otters between 1992 and 2009. The model explaining condition using the given variables gave only a moderate fit ($R^2 = 0.45$), and the relationship between otter body condition and pollutants differed between pollutants, and in some cases with sex and age class. Otter body condition decreased with increasing concentrations of PCB153 (males and females), PCB128 (in sub-adults and juveniles only, with the opposite trend for adult otters) and PCB 180 (for adult otters only, with the opposite trend for sub-adult and juvenile otters). Conversely, otter body condition increased with ppTDE and PCB138.

3.4.2 Body length

Body length was measured in 708 otters between 1992 and 2009. The model explaining body length using the given variables gave a good fit ($R^2 = 0.72$). The relationship between otter body length and pollutants differed between pollutants, and showed particularly mixed results with sex and age class. For example, increasing concentrations of PCB153, 138 and 170, and sum TEQ, were all associated to some extent with smaller body size, but only in females, adult males, juveniles, and sub-adults, respectively. When examining trends within other subgroups of the population, either the reverse was true, or no significant relationship was found.

3.5 Reproductive indicators

3.5.1 Baculum length

Baculae from 409 male otters were measured between 1992 and 2009. The model explaining baculum length using the given variables gave a good fit ($R^2 = 0.89$), but baculum length showed no significant association with any of the measured pollutants.

3.5.2 Baculum weight

Baculae from 282 male otters were weighed between 1992 and 2009. The model explaining baculum weight using the given variables gave a good fit ($R^2 = 0.96$). There was a weak but significant positive association between PCB138 and baculum weight (Figure 3), i.e. baculum weight is higher in individuals with higher concentrations of PCB138. No significant association was found between baculum weight and any other pollutant.

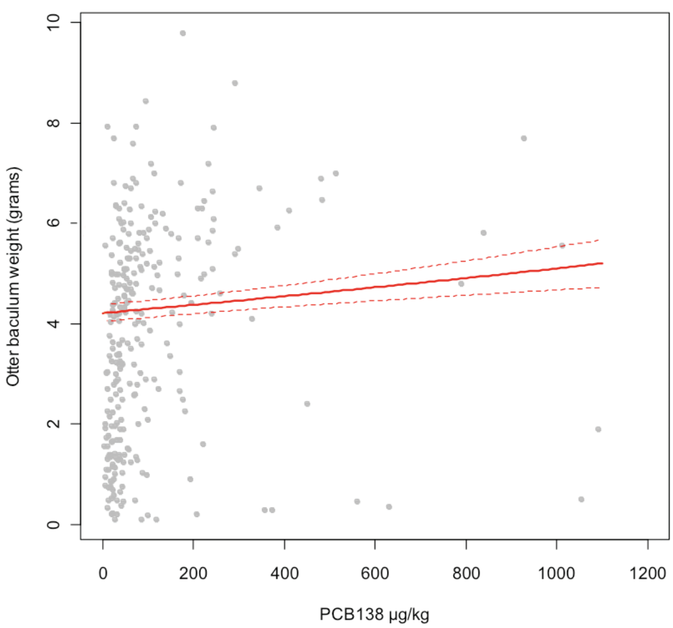


Figure 3 Relationship between pollutants and otter baculum weight (grams). Dashed lines indicate +/- 1 standard error. Raw data, plotted in the background in grey, covers the whole dataset, whereas model results are predictions where all other variables (e.g. body size) are controlled as specified in Appendix 2.

3.5.3 Testes weight (sum)

Testes from 217 male otters were weighed between 1995 and 2009 (only four of these records were before 2004). The model explaining testis weight using the given variables gave a good fit ($R^2 = 0.78$). PCB128 concentration was negatively associated with testis size (at higher concentrations, testis weight was less), but conversely ppDDE and PCB138 both show a positive association with testis size, and no other pollutants show any significant association.

3.5.4 Recent female reproduction

Presence / absence of recent reproduction was recorded in 152 adult females between 1992 and 2009; 56 were pregnant or lactating, 96 were not. The model explaining recent female reproduction using the given variables gave only a moderate fit ($R^2 = 0.35$). Recent reproduction was significantly less likely in female otters with higher PCB153 concentrations; conversely, recent reproduction was more likely in females with higher PCB187 levels and higher Sum PCB TEQs.

3.6 Parasite burden

3.6.1 Gall bladder parasites

Presence / absence of gall bladder parasites *(P. truncatum or M. albidus)* was recorded in 313 individuals between 2004 and 2009. The model explaining gall bladder parasite presence using the given variables gave a poor fit ($R^2 = 0.21$), meaning predictions should be treated with caution. Gall bladder parasites were significantly more likely to be found in otters with higher PCB128 and PCB170 concentrations, and less likely to be found in otters with higher PCB153 concentrations.

3.6.2 Toxoplasma gondii

204 otters collected between 1995 and 2008 were tested for *Toxoplasma gondii* antibodies (only six of these records were before 2004). The model explaining *T. gondii* presence using the given variables gave a poor fit ($R^2 = 0.12$), meaning predictions should be treated with caution. Although PCB congeners 138 and 153 significantly explained some variation in the incidence of *T. gondii*, large standard error around the model predictions give very low confidence in these trends and therefore they are not presented here.

3.6.3 Ectoparasites

Presence / absence of ectoparasites was recorded in 371 individuals between 2004 and 2009 (with an additional six records between 1995 and 2003). It was not possible to fit a model to the data using the otter and pollutant variables under investigation.

3.7 Organ weights

3.7.1 Adrenal glands

Adrenals from 485 otters were weighed between 1992 and 2009. The model explaining adrenal weight using the given variables gave only a moderate fit ($R^2 = 0.43$). Adrenal weight showed positive associations with concentration of PCB170 (for all age classes and both sexes of otters), and dieldrin (in female otters only). Conversely, negative associations were found between PCB187 and adrenal weight in female otters, and between dieldrin and adrenal weight in male otters (Figure 4).

3.7.2 Kidneys

Kidneys from 454 otters were weighed between 1992 and 2009. The model explaining kidney weight using the given variables gave a good fit ($R^2 = 0.75$). There was a small but significant negative association between otter kidney weight and dieldrin, regardless of otter age-class or sex. No other pollutants explained variation in kidney weight.

3.7.3 Spleen

Spleens from 328 otters were weighed between 1992 and 2009. The model explaining spleen weight using the given variables gave a good fit ($R^2 = 0.70$), but there were no associations between spleen weight and pollutant concentrations.

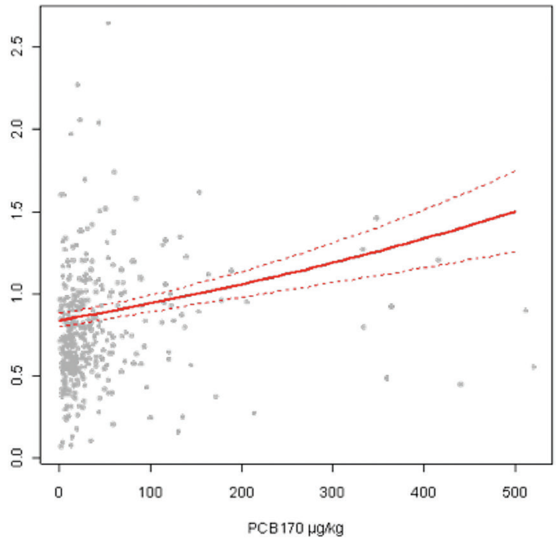
3.7.4 Heart

Hearts from 253 otters were weighed between 1992 and 2009. The model explaining heart weight using the given variables gave a good fit ($R^2 = 0.88$). Heart weight was significantly associated with concentration of many pollutants, but showed varying trends between pollutants, and in many cases differing associations dependent on sex and age class. Heart weights were significantly negatively associated with dieldrin and PCB 187 for all age-classes and both sexes (with a more pronounced association for PCB187 in juvenile and sub-adults). Negative associations were also found for DDE (adults only), TDE (juveniles only) and PCB180 (males only), but conversely, positive associations were found with DDE in juveniles and sub-adults, with TDE for sub-adults and adults, and with PCB180 for females. Positive associations were found for all age-classes and sexes with HCBenz and PCB 128 (with a more pronounced association for males than females).

3.7.5 Liver

Livers were weighed in 277 otters between 1992 and 2009. The model explaining liver weight using the given variables gave a good fit ($R^2 = 1$), but none of the pollutants were associated with otter liver weight.

a)



b) c)

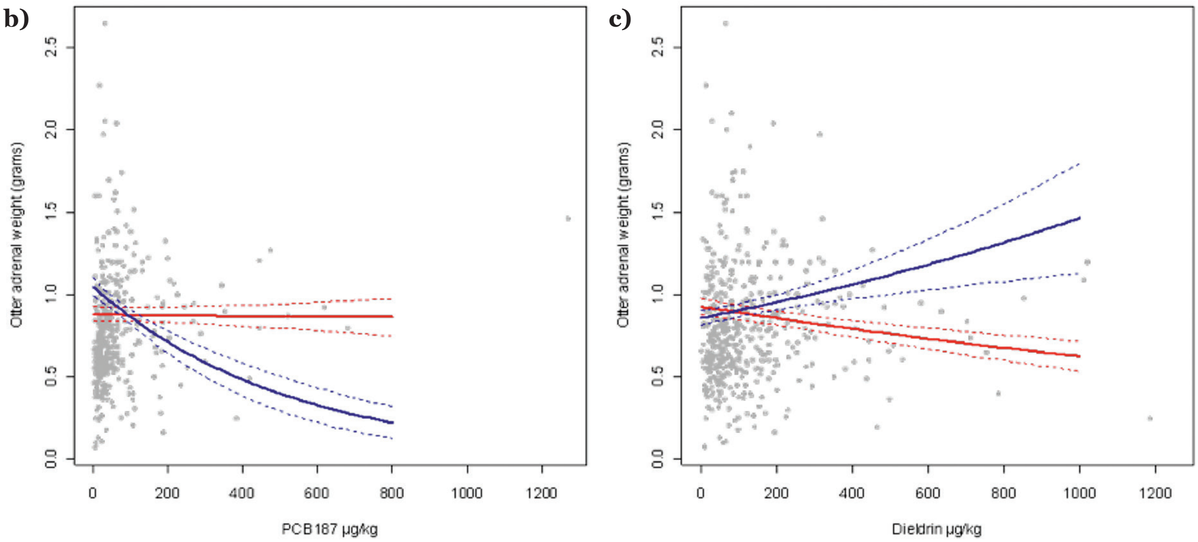


Figure 4. Relationship between adrenal weights and pollutants
a) PCB 170, red line indicates relationship for males and females. b) PCB187 and c) Dieldrin, red line indicates relationship for males, blue indicates females. Dashed lines indicate +/- 1 standard error. Raw data, plotted in the background in grey, covers the whole dataset whereas model results are controlled for age, sex etc.

3.8 Abnormalities

3.8.1 Kidney stones

Kidneys were examined for stones in 673 otters between 1992 and 2009, and were found in 15% of otters. The model explaining presence of kidney stones using the given variables gave a poor fit ($R^2 = 0.13$), meaning predictions should be treated with caution. The probability of having kidney stones increased significantly with PCB153 levels for all otters. No other pollutants were associated with kidney stones.

3.8.2 Fighting injuries

It was possible to assess 668 carcasses for fighting injuries between 1992 and 2009, and they were found in 16% of otters. The model explaining presence of fighting injuries using the given variables gave a poor fit ($R^2 = 0.17$), meaning predictions should be treated with caution. There were no associations between the presence of fighting injuries and pollutants.

3.9 Uncommon abnormalities

The following abnormalities recorded in otters are uncommon so it was not possible to statistically test for any associations with pollutants. Notable cases are described.

3.9.1 Cryptorchidism

In normal foetal development testes descend through the abdominal wall before birth. Cryptorchidism is a condition where the testes are not descended, and remain abdominal. Only five cases of cryptorchidism have been found in over 2000 otters examined at Cardiff University Otter Project to date, so too few data are available to test for change with time, but it is noteworthy that none were seen prior to 2008. Three of these (two cases of bilateral and one case of unilateral cryptorchidism) have accompanying liver toxicology data (otter number 1134, 1166 and 1345); the liver of one other was too badly damaged (otter number 1160) and the liver of another is yet to be analysed (otter number 1869). In two of these cases, levels of some pollutants (dieldrin in one otter and PCBs 170, 180 and Sum PCB TEQ in the other otter) were above the upper quartiles of the data distribution, but there are too few data to test whether there is any significant association.

3.9.2 Cysts and swellings

Cysts or swellings likely to have clinical significance (not including small swellings likely to be associated with scarring, e.g. on the feet) were found in 9 cases (Table 6). Most of these had some pollutant levels above average or above the upper quartile (Table 6), but there was no clear pattern to suggest any particular pollutant was associated with cysts or swellings. One otter (911) had a small fluid filled cyst on the vas deferens to the left testicle. Since the last otter in this report was found (2009), over 600 more otter carcasses have been examined: cysts on the vas deferens are now much more common, occurring in 11% of adult male otters examined.

3.9.3 Infection

Between 1992 and 2009 only 41 out of 755 otters (5%) showed signs of infection. Although most of these (37) had some pollutant levels above average or above the upper quartile (Table 7), there were no clear trends with any particular pollutant.

Table 6. Details of cysts and swellings found in otters at post mortem examination

Otter number	Description of abnormality	Pollutants n above average (n above upper quartile)
911	Small fluid filled cyst on the left vas deferens.	11(11)
792	Small solid lump on left adrenal.	1(0)
870	Soft yellow lump adhering to surface of liver, 0.25g.	11 (6)
1051	Swollen lump at thyroid.	1(0)
1111	Fatty lump on right ovary.	0(0)
1158	Multiple hard cyst-like objects in all lobes of lung.	10 (3)
1182	Hardened granular lumps throughout lungs, most severe in apical lobes	9 (7)
1258	Large firm swelling to underside of throat.	8 (2)
1282	Several small cysts in lungs.	8 (1)

Table 7. Details of infections found in otters at post mortem examination

Otter number	Description of abnormality	Pollutants n above average (n above upper quartile)
13	Oral and respiratory infections.	0
101	Respiratory (lower) infection	5(5)
255	Respiratory infection	11(10)
301	Urethra blocked, possible urinary infection	11(11)
480	Condition suggestive of death by infection.	7(2)
552	Oral infection, with evidence of decay in upper incisors and a gum infection.	10(4)
638	Occipital bone over left eye broken and left eye badly damaged. Infection extended into nasal cavities.	3(0)
650	Cause of death probably secondary infection following fighting injuries.	6(6)
688	Infection arising from fighting injury. Also respiratory infection, probably secondary to infection on head.	10(8)
717	Extensive white nodular lesions on lobes of lungs, appear to be surface only.	8(3)
792	Small solid lump on left adrenal.	1(0)
810	Large pus filled infection (main area 14cm along) extending along flank from front of the ribcage to the spine.	9(3)
850	Adrenal glands slightly enlarged, spleen very enlarged.	8(7)
879	Grossly enlarged retropharyngeal lymph nodes - photo taken. Likely response to an infection.	11(5)
908	Infection following shot to left flank	6(0)
932	Infection arising from fighting injuries, especially to scrotal area.	6(2)
946	Severe respiratory infection.	10(7)
963	Bacterial infection of the liver.	9(7)
964	Lower right teeth missing and root still slightly open with some infection	11(4)
1005	Hind right foot swollen - oedematous tissue. Severe infection, pre-dates RTA.	7(3)
1017	Respiratory infection.	6(3)
1020	Severe respiratory infection.	7(4)
1042	Respiratory infection.	8(2)
1053	Slight infection to ducts of hind right nipple	10(1)
1060	Severe bacterial infection of lungs	11(10)
1080	Tooth infection	10(5)
1081	Respiratory infection.	9(6)
1087	Possible liver infection	6(0)
1089	Severe respiratory infection	0
1115	Possible respiratory infection	8(4)
1126	Severe respiratory infection.	8(1)
1145	Puncture wound behind R ear - swollen tissue, signs of infection.	10(6)
1147	Infection of UL canine, also possible respiratory infection	10(7)
1182	Severe lung infection	9(7)

4. Discussion

Of seventeen health indicators analysed, eleven show some association with concentration of at least one pollutant. Raw data are, however, highly variable, and interpretation is complex as trends vary not only between pollutants but also in many cases with age and sex. As expected, factors such as otter age, sex, size etc. are associated with health indicators; by taking a predictive modelling approach these additional variables were statistically controlled, in order to focus specifically on associations with pollutants.

Most of the potential health indicators examined showed no change over time. Over the same period, otter populations have increased, suggesting that the otter population is largely healthy. While it is recognised that sampling using largely road killed otters may be biased towards the healthy part of the otter population, this does not detract from the value of using such data to test for associations between health and pollutants.

Negative impacts on health (e.g. due to pollutants) might be expected to result in a loss of body condition, or failure to achieve normal growth. In the current study however, while negative associations were found with some pollutants (higher concentrations associated with reduced condition or length), the converse was true for other pollutants, or in other groups of the otter population (e.g. with sex or age). Condition is a crude indicator of health, and body size is driven by a wide range of variables including sex, age, diet and genetics. Lipophilic pollutants tend to be associated with high fat prey; otters with an abundant, high quality diet are therefore likely to be exposed to higher pollutant loading, while also likely to be in good condition and able to achieve high growth rates. These two opposing drivers may explain the conflicting results seen in the current analysis.

A wide range of evidence suggests that POPs can have negative effects on reproductive systems (see Vos et al., 2000 for review; for example the effect of DDT and dieldrin on sparrowhawk reproduction, Porter and Wiemeyer, 1969). Using data collected post mortem from frozen carcasses restricts the nature of reproductive indicators that can be measured, but relatively crude measures such as baculum size have previously been associated with pollutant levels in a range of species (for example polar bears, Sonne et al, 2006), including otters (e.g. a negative association between baculum length and levels of both dieldrin and DDT derivatives in juvenile otters, Simpson, 2007). Associations between indicators of reproductive health and the measured pollutant concentrations in the current study were, however, inconsistent. Baculum length showed no association with any of the measured pollutants (contrary to Simpson, 2007), while baculum weight showed a positive association with one PCB congener (PCB138). Testis weight showed a mix of positive and negative associations with differing pollutants, as did the likelihood of recent reproduction in female otters.

In the current study, baculum weight (but not length) declined over time. Furthermore, reproductive abnormalities in males, including cryptorchidism and cysts on the vas deferens have been recorded more frequently in recent years than previously, although numbers are too few to include in statistical analyses. Both cryptorchidism and cysts on the vas deferens have been linked to exposure to pollutants, particularly those with hormone disrupting properties. For example, laboratory animal studies have shown that in utero exposure to xeno-oestrogens and anti-androgenic chemicals (that is man-made chemicals which mimic oestrogen or block androgen function) can cause reproductive problems including cryptorchidism, and there is substantial evidence of this occurring in wildlife (reviewed by Skakkebaek et al., 2001). Cysts on the vas deferens were also recently found in otters in Sweden (e.g. Roos and Agren, 2011). The cause of these cysts is unclear, but the authors (Roos and Agren 2011) suggest it may be due to foetal exposure to high levels of oestrogenic compounds, which hinder the normal regression of the Müllerian ducts during development, resulting in vestigial remnants which become encysted. The decline in baculum weight over time, and the possible increase in reproductive abnormalities in the current study, give cause for concern, and continued monitoring should receive high priority. The pollutants analysed in the current report do not appear to show any consistent negative associations with the selected reproductive indicators, suggesting that other factors (potentially including newer pollutants that are not measured here) are driving the observed changes.

Poor health can be associated with increased parasite burden, both through cause and effect (i.e. high parasite load can have a negative impact on health, and ill health can lead to increased susceptibility to infection). In the current study, however, the incidence rates of parasitic infection were poorly explained by the modelled variables (including pollutants), allowing limited confidence in conclusions drawn from those models. It is likely that factors other than those examined in the current study, such as habitat, alternative host distribution, climate (Sherrard-Smith, Chadwick and Cable, 2012), or other pollutants not tested in the current study, better explain parasite presence.

Exposure to pollutants can sometimes cause change in organ mass (e.g. Chaturvedi, 1993). In the current study, neither liver nor spleen mass (controlled for otter size) showed any significant associations with the measured POPs, but a number of significant associations were found with adrenal, kidney and heart mass. These associations, however, showed no consistent trends between pollutants or subgroups of the population. Of the pollutants measured, dieldrin showed the most consistent significant associations, being negatively associated with kidney and heart weight across sex and age groups, and negatively associated with adrenal weight in males (although positively associated in females).

Although declines in most of the measured POPs have coincided with temporal changes in adrenal weight, baculum weight, and the incidence of fighting injuries, there are no consistent statistical associations between concentrations of POPs and the measured health indicators. It is therefore likely that temporal change in other environmental variables, not included in the current analyses, are driving those changes. Adrenals glands become enlarged in response to physiological stress, so a gradual decline over time, as found in the current study, might suggest improvements in population health. A decrease in baculum weight may be regarded as a negative response to environmental change, although the functional significance of this is not known. Given the potential role of endocrine disrupting chemicals in reproductive health, monitoring of the levels of such chemicals in biota is clearly merited, as is the monitoring of reproductive health parameters as indicators of exposure to endocrine disruptors. The increased occurrence of fighting injuries could be due to higher densities of otters (population size has increased over the study period), but fighting injuries were not significantly more common in regions such as the South West, where otters are more common. A more detailed analysis including data on the extent and severity of injuries may more clearly reveal associations.

5. Key recommendations

- Regular monitoring of pollutants in top predators should be continued, and as a top predator of aquatic systems in the UK, the otter is an ideal sentinel for pollutants. Although implementation of the Water Framework Directive should ensure improved water quality in the UK, it does not currently include any requirement to monitor pollutants in top predators.
- While continuing to monitor 'historical' (banned) pollutants is important in case of recirculation or illegal usage, it is also important to regularly revise the suite of pollutants measured, and ensure that currently used chemicals of concern are included. Failure to do so may lead to a false sense of security and cause emerging threats to be missed.
- The range of pollutants currently under surveillance in top predators should be expanded, and funding should be made available to achieve this. Additions might include (i) chemicals already listed on the Stockholm Convention, that are not currently monitored in otters (e.g. perfluorooctane sulphonic acid (PFOS), its salts and perfluorooctane sulphonyl fluoride (PFOS-F)), (ii) others which are sufficiently persistent and bioaccumulative to be of very high concern, but have not yet been proposed for listing or would not meet all the criteria (e.g. musk xylene), and (iii) non highly bioaccumulative pollutants which may also affect health, particularly including contaminants with endocrine disrupting properties (eg. certain phthalates, bisphenol A, 4-tertiary-octylphenol).
- Monitoring of otter health should be continued, particularly in light of the potential changes observed in male otter reproductive health. Additional, more sensitive, indicators should be sought to give a broader picture of otter health. In particular, indicators of endocrine disruption should be included.
- The impact of multiple pollutants on animals is poorly understood and should be assessed in addition to analysis of single pollutants.
- There is a need to keep abreast of current health trends in wildlife so that early health warnings can be acted on before population level effects manifest. In order to protect biodiversity it is imperative to monitor health indicators in several species that, together, can act as sentinels for the freshwater, marine and terrestrial environments.
- Greater coordination between those monitoring the environment and human health (e.g. through continuation and expansion of the WILDCOMS initiative www.wildcoms.org.uk) would enable a more forward looking approach to identify emerging threats.

6
References

Bradshaw, A.V. and Slater, F.M. (2002). *A postmortem study of otters (Lutra lutra) in England and Wales.* Environment Agency R&D Technical Report W1-019/TR.

Chadwick, E.A. (2007). *Post mortem study of otters in England and Wales 1992-2003.* Environment Agency Science Report: SC010065/SR.

Chanin, P. (2003). *Ecology of the European Otter.* Conserving Natura 2000 Rivers Ecology Series No. 10. English Nature, Peterborough.

Chaturvedi, A.K. (1993). *Toxiological evaluation of mixtures of 10 widely used pesticides.* Journal of Applied Toxicology, 13 (3), 183-188.

Crawford, A., Evans, D., Jones, A. and McNulty, J. (1979) *Otter survey of Wales 1977-78.* Society for the Promotion of Nature Conservation, Nettleham.

Crawford, A. (2010). *Fifth otter survey of England 2009-2010.* Bristol, Environment Agency.

Houde, M., Martin, J.W., Letcher, R.J., Solomon, K.R. and Muir, D.C.G. (2006). *Biological monitoring of polyfluoroalkyl substances: A review.* Environmental Science & Technology, 40(11) 3463-3473.

Kean, E.F. and Chadwick, E.A. (2012). *Persistent organic pollutants in the livers of Eurasian otters from England and Wales collected between 1992 and 2009.* Cardiff University Otter Project report to the Environment Agency.

Kortenkamp, A. (2007). *Introduction: Endocrine Disruptors—Exposure Assessment, Novel End Points, and Low-Dose and Mixture Effects.* Environmental Health Perspectives. 2007 December; 115(S-1): 7).

Kruuk, H. and Conroy, J.W.H. (1991). *Mortality of otters Lutra lutra in Shetland.* Journal of Applied Ecology, 28, 83-94.

Lenton, E.J., Chanin, P.R.F. and Jefferies, D.J. (1980). *Otter Survey of England 1977-79.* London: Nature Conservancy Council.

Porter, R.D. and Weimeyer, S.N. (1969). *Dieldrin and DDT – effects on sparrowhawk eggshells and reproduction.* Science, 165(3889), 199-200.

Richards, N.L., Cook, G., Simpson, V., Hall, S., Harrison, N. and Scott, K.S. (2001). *Qualitative detection of the NSAIDs diclofenac and ibuprofen in the hair of Eurasian otters (Lutra lutra) occupying UK waterways with GC-MS.* European Journal of Wildlife Management, 57:1107-1114.

Roos, A. and Agren, E. (2011). *Müllerian duct cysts on the vas deferens in Swedish wild otters (Lutra lutra). in Prigioni et al.(eds) 2011.* Abstracts XIth International Otter Colloquium, Hystrix It. J. Mamm., (n.s.) Supp. (2011): 1-122.

Sherrard-Smith, E., Chadwick, E.A. and Cable, J. (2012). *Abiotic and biotic factors associated with tick population dynamics on a mammalian host: Ixodes hexagonus infesting otters, Lutra lutra.* PLoS One doi:10.1371/journal.pone.0047131.

Simpson, V.R. (1998). *A post mortem study of otters (Lutra lutra) found dead in South West England.* Environment Agency R&D Technical Report W148.

Simpson, V.R. (2007). *Health status of otters in southern and south west England 1996-2003.* Environment Agency Science Report: SC010064/SR1.

Skakkebæk, N.E., Rajpert-De Meyts, E. and Main K.M. (2001). *Testicular dysgenesis syndrome: an increasingly common developmental disorder with environmental aspects.* Human Reproduction, 16 (5): 972-978.

Sonne, C., Leifsson, P.S., Dietz, R., Born, E.W., Letcher, R.J., Hyldstrup, L., Riget, F.F., Kirkegaard, M. and Muir, D.C.G. (2006). *Xenoendocrine pollutants may reduce size of sexual organs in East Greenland polar bears (Ursus maritimus).* Environmental Science & Technology, 40(18), 5668-5674.

Strachan, R. (2010). *Otter survey of Wales 2009-2010 Technical Summary.* Environment Agency Wales, Cardiff.

Vos, J.G., Dybing, E., Greim, H.A., Ladefoged, O., Lambre, C., Tarazona, J.V., Brandt, I. and Vethaak, A.D. (2000). *Health effects of endocrine-disrupting chemicals on wildlife, with special reference to the European situation.* Critical reviews in Toxocology, 30(1), 71-133.

Walker, L.A., Moeckel, C., Pereira, M.G., Beith, S.J., Potter, E.D., Chadwick, E.A., & Shore R.F. (2012). *Polybrominated Diphenyl Ethers (PBDEs) in Eurasian otters (Luta lutra) collected from Britain in 2010: a Predatory Bird Monitoring Scheme (PBMS) Report.* Centre for Ecology & Hydrology, Lancaster, 14pp

Walker, L.A., Lawlor, A.J., Chadwick, E.A., Potter, E., Pereira, M.G. & Shore, R.F. 1 (2011). *Inorganic elements in the livers of Eurasian otters, Lutra lutra, from England and Wales in 2009 - a Predatory Bird Monitoring Scheme (PBMS) report.* Centre for Ecology & Hydrology, Lancaster, UK.

Appendix 1.

Pollutants measured in otters

Pollutants measured in otter livers by the Environment Agency between 1992 and 2009.

Pollutant	Listed under the Stockholm convention (www.pops.int)	Included in current analysis (13 most frequently detected)
a-HCH	Yes	
b-HCH	Yes	
g-HCH	Yes	
d-HCH		
Dieldrin	Yes	Yes
Aldrin	Yes	
Isodrin	Isomer of Aldrin	
Endrin	Yes	
DDE-OP	Derivative of DDT	
DDE-PP	Derivative of DDT	Yes
DDT-OP	Yes	
DDT-PP	Yes	
TDE-OP	Derivative of DDT	
TDE-PP	Derivative of DDT	Yes
PCB 8	Yes	
PCB 18	Yes	
PCB 20	Yes	
PCB 28	Yes	
PCB 31	Yes	
PCB 35	Yes	
PCB 44	Yes	
PCB 52	Yes	
PCB 66	Yes	
PCB 77	Yes	
PCB 101	Yes	
PCB 105	Yes	Yes, as part of TEQ
PCB 118	Yes	Yes, as part of TEQ
PCB 126	Yes	
PCB 128	Yes	Yes
PCB 138	Yes	Yes
PCB 149	Yes	
PCB 153	Yes	Yes
PCB 156	Yes	Yes, as part of TEQ
PCB 169	Yes	
PCB 170	Yes	Yes
PCB 180	Yes	Yes
PCB 187	Yes	Yes
HCB	Yes	Yes

A further 22 pollutants (atrazine, simazine, trifluralin, malathion, parathion ethyl, parathion methyl, fenitrothion, endosulphan A and B, araclor, hexachlorobutadiene, trichlorobenzene 123, 124 and 135, and metals Zn, Ni, As, Hg, Pb, Cr, Cu and Cd [Chadwick, 2007; Kean and Chadwick, 2012]) were tested for previously by the Environment Agency but discontinued following initial results in the early 1990s (most were below detection limits). Copies of reports can be obtained by contacting the Otter Project www.otterproject.cf.ac.uk.

Polybrominated diphenyl ethers and other flame retardants (BDEs 17, 28, 30, 32, 35, 37, 47, 49, 51, 66, 71, 77, 85, 99, 100, 118, 119, 126, 128, 138, 153, 154, 183, 190, 196 and 197, pentabromomethylbenzene, pentabromoethylbenzene, hexabromobenzene, hexabromocyclododecane, dechlorane plus 1, dechlorane plus 2 [Walker et al, 2012]) and inorganic elements (Al, Sb, As, Cd, Cr, Co, Cu, Fe, Mn, Hg, Mo, Ni, Se, Sr, and Zn [Walker et al, 2011]) have been measured in otter livers through a collaboration between Cardiff University Otter Project and the Predatory Bird Monitoring Scheme (PBMS) at the Centre for Ecology & Hydrology (CEH). Reports can be downloaded directly from https://wiki.ceh.ac.uk/display/pbms/Home.

Appendix 2.

Statistical analyses

Model error distributions and link functions were selected based on a priori considerations (e.g. a binomial error distribution for binomial data) or the Akaike Information Criteria (AIC). Manual backwards-stepwise model refinement was conducted to remove non-significant terms from each model. The variables and interactions included in each initial model are shown in the table below. Sex and age-class were not included in models investigating a health indicator only relevant to one age or sex e.g. recent reproduction applies only to adult females.

To assess the significant associations between health indicators and pollutants, model predictions and standard error of predictions were calculated while controlling for all other significant variables in the relevant model. Variables were controlled as follows: where controlling for sex and age, predictions were based on adult male otters (the most common group in the dataset). Where controlling for Region, predictions were based on Wales, where controlling for season, predictions were based on winter (the most numerous groups, and likely to return conservative estimates of pollutant levels). Where controlling for year, length, and condition the mean was used. Where controlling for cause of death and carcass condition "RTA", and "Fresh" (the most common categories) were selected respectively.

Terms initially included in models		
Independent variables		Interactions
Sex (f)		Sex: pollutants
Age-class (f)		Age-class: pollutants
Length (c)		
Condition (c)		
Region (f)		
Year of death (c)		
Season (f)		
Carcass condition (f)		
Cause of death (f)		
Pollutants:	Dieldrin ppDDE ppTDE PCB128 PCB138 PCB153 PCB170 PCB180 PCB187 HCBenz Sum PCB TEQ	
c= continuous variable, f= categorical variable		

Appendix 3.

Variables in generalised linear models explaining variation in otter health indicators

		Dependent variables							
		Size		Reproduction				Parasites	
		Condition [gi]	Body length [gi]	Baculum length [gi]	Baculum weight [gi]	Testes weight [gi]	Recent female reproduction [blt]	Gall bladder parasites [bc]	Toxoplasma [blt]
	Sample size	679	708	409	282	217	152	313	204
	GLM R-squared[1]	0.45	0.72	0.89	0.96	0.78	0.35	0.21	0.12
Pollutant variables	Dieldrin								
	Dieldrin:Age						NA		
	Dieldrin:Sex			NA	NA	NA	NA		
	ppDDE					4.49*			
	ppDDE:Age						NA		
	ppDDE:Sex			NA	NA	NA	NA		
	ppTDE	6.12*							
	ppTDE:Age						NA		
	ppTDE:Sex			NA	NA	NA	NA		
	PCB128					3.96*		5.50*	
	PCB128:Age	7.42***					NA		
	PCB128:Sex			NA	NA	NA	NA		
	PCB138	4.78*			5.17*	5.02*			
	PCB138:Age						NA		10.52**
	PCB138:Sex		16.26***	NA	NA	NA	NA		
	PCB153						11.45***	11.80***	
	PCB153:Age		5.84**				NA		10.71**
	PCB153:Sex	5.46*	10.81**	NA	NA	NA	NA		
	PCB170								
	PCB170:Age		6.01**				NA		
	PCB170:Sex			NA	NA	NA	NA		
	PCB180								
	PCB180:Age	5.48**					NA		
	PCB180:Sex			NA	NA	NA	NA		
	PCB187						6.57*		
	PCB187:Age		16.21***				NA		
	PCB187:Sex			NA	NA	NA	NA		
	HCBenz								
	HCBenz:Age						NA		
	HCBenz:Sex			NA	NA	NA	NA		
	SumPCB-TEQ						7.38**		
	SumPCB-TEQ:Age		8.90***				NA		
	SumPCB-TEQ:Sex			NA	NA	NA	NA		
Other independent variables	Year				20.55***				
	Season	4.78*					11.0662*		
	Sex			NA	NA	NA	NA		
	Age class			189.26***	75.80***	67.69***	NA	15.71***	
	Condition	NA		45.71***		24.83***			
	Body length	11.87*	NA	290.72***	66.40***	91.95***	6.064*		
	Region	3.57***			3.38**	2.47*	17.53*	31.57***	14.09*
	Carcass condition						11.66***		
	Cause of death	15.20***							

F value (Chi square for binomial models) and significance for each term *p<0.05, **p<0.01, ***p<0.001. All significant associations are highlighted in blue. Blank cells = term was not significant in the minimal model. NA = term was not included in the model. Red = low R^2 so caution should be taken in interpretation of the results; green = high R2 and high confidence in results; amber = medium R^2.

[1] pseudo R^2 for binomial models, g=gaussian GLM, b=binomial GLM, i=identity link, l=log link, lt=logit link, c=cloglog link

	Organ weights					Abnormalities	
	Adrenals [gi]	Kidney s [gi]	Spleen [gi]	Heart [gi]	Liver [gl]	Kidney stones [bc]	Fight [bc]
Sample size	485	454	328	253	277	673	668
GLM R-squared*	0.43	0.75	0.70	0.88	1.00	0.13	0.17
Dieldrin		6.84**		8.03**			
Dieldrin:Age							
Dieldrin:Sex	11.40***						
ppDDE							
ppDDE:Age				4.88**			
ppDDE:Sex							
ppTDE							
ppTDE:Age				3.15*			
ppTDE:Sex							
PCB128							
PCB128:Age							
PCB128:Sex				4.78*			
PCB138							
PCB138:Age							
PCB138:Sex							
PCB153						5.07*	
PCB153:Age							
PCB153:Sex							
PCB170	12.12***						
PCB170:Age							
PCB170:Sex							
PCB180							
PCB180:Age							
PCB180:Sex				9.04**			
PCB187							
PCB187:Age				6.16**			
PCB187:Sex	16.60***						
HCBenz				7.65**			
HCBenz:Age							
HCBenz:Sex							
SumPCB-TEQ							
SumPCB-TEQ:Age							
SumPCB-TEQ:Sex							
Year	27.24***						14.41***
Season	3.57*	3.00*					
Sex		16.31***	27.80***		13.58***	6.10*	23.52***
Age class	15.44***	20.59***	4.37*			27.05***	20.61***
Condition	12.36***	109.84***	80.45***	8.28**	69.70***		
Body length	63.25***	191.10***	150.02***	149.92***	181.92***	4.61*	
Region				4.19***	4.01***		
Carcass condition							
Cause of death	3.93**			4.09***			55.80***

CHEM Trust

Protecting humans and wildlife from harmful chemicals

All CHEM Trust briefings and reports can be downloaded from **www.chemtrust.org.uk**

Previous publications include:

i) *What could new EU chemicals legislation deliver for public health?* outlining the health benefits that the new EU Regulation (REACH) could provide (2007).

ii) *Chemicals compromising our children* – a review of the potential damage chemicals may cause to the developing brain (2007).

iii) *Breast cancer and exposure to hormonally active chemicals: An appraisal of the scientific evidence* – a report for medical professionals and scientists by Professor Andreas Kortenkamp of the London School of Pharmacy (2008).

iv) *Factors influencing the risk of breast cancer – established and emerging* – a briefing for the public on the potential role of chemicals in breast cancer (2008).

v) *Breast cancer: Preventing the preventable* – a leaflet for the public.

vi) *Effects Of Pollutants On The Reproductive Health Of Male Vertebrate Wildlife – Males Under Threat* by Gwynne Lyons, showing that males from each of the vertebrate classes, including bony fish, amphibians, reptiles, birds and mammals, have been feminised by chemicals in the environment (2008). A summary, in German, was published in 2009 by BUND (FOE Germany).

vii) *Male reproductive health disorders and the potential role of exposure to environmental chemicals* by Professor Richard Sharpe of the Medical Research Council (2009).

viii) *Men under threat: The decline in male reproductive health and the potential role of exposure to chemicals during in-utero development* – a fully referenced briefing by Gwynne Lyons (2009).

ix) *Men under Threat* – a leaflet for the public (2009).

x) *Why mollusc toxicity tests for endocrine disruptors and other chemicals are needed* - A briefing for policy makers re testing chemicals for their toxic properties (2009).

xi) *A review of the role pesticides playin some cancers: Children, farmers and Pesticides users at risk?* by Gwynne Lyons and Professor Andrew Watterson (2010).

xii) *Chemical cocktails - harmful mixtures upset our hormones* by CHEM Trust, HEAL and WWF (2010).

Xiii) *Review of the science linking chemical exposures to the human risk of obesity and diabetes,* by Professor Miquel Porta and Professor Duk-Hee Lee (2012)

Some of these documents are available in Russian, Polish, Czech, Italian, Spanish, French, German and Slovenian.

www.chemtrust.org.uk

PRINT GUY

This report was designed and printed February 2013 by Printguy:
www.printguy.co.uk
on 100% recycled paper using vegetable-based inks