Elevated Levels of Organohalogen Contaminants in White-Bellied Sea-Eagles (Haliaeetus Leucogaster) from Sydney, Australia

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In 2001, the Australian Government established the National Dioxins Program that involved a range of studies measuring emissions from sources such as bushfires, as well as dioxin levels in the environment, food and population. One such study on the aquatic environment detected dioxin-like chemicals in all Australian aquatic sediments analysed, with middle bound concentrations ranging from 0.002 to 520 pg TEQ g^{-1} dry matter (dm). Highest concentrations were found in the sediments sampled from the Parramatta River estuary (100 and 520 pg TEQ g^{-1} dm) and the western section of Port Jackson (78 and 130 pg TEQ g^{-1} dm), in close proximity to historical manufacturing point sources around Homebush Bay.[1] One site in particular manufactured a range of chemicals from 1928 to 1986 including the herbicides 2,4-D and 2,4,5-T plus chlorophenol timber preservatives. In the Spring of 2004, a breeding pair of White-bellied Sea-eagles ***(Haliaeetus leucogaster)***were discovered deceased within days of each other in the area of Homebush Bay with no apparent infectious/parasitic diseases or trauma that appeared to be responsible.[2] White-bellied Sea-Eagles are a common sight in coastal and near coastal areas of Australia. Birds form permanent pairs that inhabit territories throughout the year. They feed mainly off aquatic animals, such as fish, but they take other birds and mammals as well. An investigation of the levels of dioxins (PCDDs/PCDFs), dioxin-like polychlorinated biphenyls (DL-PCBs) and polybrominated diphenyl ethers (PBDEs) on selected tissues from the dead birds was instigated.

Materials and methods

During post mortem examination, individual samples of brain, liver and breast muscle were removed from both the male and female birds and frozen prior to sending to our laboratory. Appropriate wildlife permits allowing scientific research were in place.

PCDD/PCDF, DL-PCB and PBDE Analyses

Standards were all purchased from Wellington Laboratories (Ontario, Canada) and were used for calibration, quantification and determination of recovery of PCDD/PCDF, DL-PCBs and PBDEs. Solvents were purchased as pesticide-quality standard and used as received. All chromatographic columns were purchased from Fluid Management Systems. (Waltham, MA, USA) and were used without any further treatment. They comprised multi-layer (basic/neutral/ acidic) silica and basic alumina and carbon (AX 21 dispersed on celite).

Sample Preparation

All tissue samples were digested overnight with concentrated hydrochloric acid. Extraction of the lipid was then performed three times using dichloromethane:hexane (25:75). Approximately 1-5g of the extracted lipid was spiked with a known quantity of $^{13}C_{12}$ surrogates and analysed by isotope dilution HRMS for the twenty-four PBDEs, seventeen 2,3,7,8-substituted PCDD/PCDFs and twelve dioxin-like PCB congeners for which WHO TEF factors have been assigned.[3] The detailed analytical methods have been previously described[4],[5]and are based upon USEPA Methods 1613, 1668 and 1664, for PCDD/Fs, PCBs and PBDEs respectively.

PCDD/PCDF, DL-PCB and PBDE Analyses

The levels of PCDDs/PCDFs and dioxin-like PCBs were calculated on a lipid weight basis (pg/g) using the avian toxic equivalency factors (TEFs)[3] to calculate the TEQs for WHO_{Avian}-TEQ. Recoveries of the $^{13}C_{12}$ PCDD/PCDF and $^{13}C_{12}$ PCB internal standards added prior to extraction and carried throughout the clean-up/fractionation steps across all tissue types averaged 74.5 ± 12.0% and 69.5 ± 13.5%, respectively. Concentrations of native analytes for PCDDs/PCDFs and PCBs were corrected for the recovery of these internal standards and middle-bound concentrations were calculated assuming that all values of the different congeners less than the limit of determination are equal to one-half the limit of determination. PBDE congeners tested were BDE17, BDE28, BDE47, BDE49, BDE66, BDE71, BDE77, BDE85, BDE99, BDE100, BDE119, BDE126, BDE138, BDE153, BDE154, BDE156, BDE183, BDE184, BDE191, BDE196, BDE197, BDE206, BDE 207, BDE 209 and levels were also calculated on a lipid weight basis (ng/g). Recoveries of the $^{13}C_{12}$ PBDE internal standards added prior to extraction and carried throughout the clean-up/fractionation steps across all tissue types averaged 68.9 ± 44.8%. Concentrations of native analytes for PBDEs were corrected for the recovery of these internal standards. Only those congeners detected at levels greater than three times the level found in the procedural blanks and that passed retention time and ion ratio quality assurance criteria were reported.

Results and Discussion.

The levels of PCDDs/PCDFs and dioxin-like PCBs have been calculated on a lipid weight basis (pg/g) using the avian toxic equivalency factors (TEFs)[6] to calculate the TEQ's for WHO_{Avian}-TEQ. Recoveries of the $^{13}C_{12}$ PCDD/PCDF and $^{13}C_{12}$ PCB internal standards added prior to extraction and carried throughout the clean-up/fractionation steps across all tissue types averaged 74.5 ± 12.0% and 69.5 ± 13.5%, respectively. Concentrations of native analytes for PCDDs/PCDFs and PCBs were corrected for the recovery of these internal standards and middle-bound concentrations were calculated assuming that all values of the different congeners less than the limit of determination are equal to one-half the limit of determination. PBDE congeners tested were BDE17, BDE28, BDE47, BDE49, BDE66, BDE71, BDE77, BDE85, BDE99, BDE100, BDE119, BDE126, BDE138, BDE153, BDE154, BDE156, BDE183, BDE184, BDE191, BDE196, BDE197, BDE206, BDE 207, BDE 209 and levels were also calculated on a lipid weight basis (ng/g). Recoveries of the $^{13}C_{12}$ PBDE internal standards added prior to extraction and carried throughout the clean-up/fractionation steps across all tissue types averaged 68.9 ± 44.8%. Concentrations of native analytes for PBDEs were corrected for the recovery of these internal standards. Only those congeners detected at levels greater than three times the level found in the procedural blanks and that passed retention time and ion ratio quality assurance criteria were reported. The concentrations of PCDDs/PCDFs, DL-PCBs and PBDEs determined are shown in Table 1.

The congener profiles for the PCDDs/PCDFs and DL-PCBs while different in the various tissues samples did not appear to be have significant variations between the male and female birds. The BDE profiles are however quite different between the male and female tissues as depicted in Figures 1-3. We were aware that the female bird prior to its death had laid a small clutch of eggs, none of which had hatched but do not know whether this has had an impact on the level or distribution of BDEs in the different tissues. While it is known the male and female birds were a breeding pair and therefore inhabited the same location, anecdotal evidence suggests that the male bird may have had a larger roaming area than the female resulting in exposure to different sources of BDEs. The bird's home base was approximately 12km west of Sydney, which is Australia's largest city having a population greater than 4 million. It is clear that it is extremely difficult to draw many conclusions from such a small sample size.

Table 1. Concentrations of PCDDs/PCDFs, DL-PCBs and PBDEs in lipid (lw) of White-bellied Sea-Eagle tissue samples from Sydney, Australia

	PCDDs pg TEQ/g lw	PCDFs pg TEQ/g lw	Non-ortho PCBs pg TEQ/g lw	Mono-ortho PCBs pg TEQ/g lw	ΣPBDEsng/g lw	% Lipid Content
Male						
Brain	13,310	180	2,790	360	2,030	7.6

Breast muscle	76,930	5,890	40,310	3,460	45,900	62.5
Liver	46,040	4,620	12,860	1,210	8,610	4.5
Female						
Brain	7,450	85	1500	175	890	6.6
Breast muscle	55,670	2,690	27,100	1,960	7,500	58.4
Liver	21,460	1,625	6,450	585	2,390	4.7

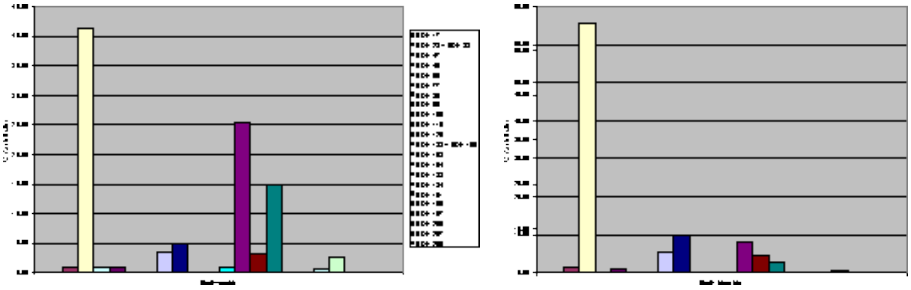

Figure 1 BDE Congener profile White-bellied Sea-eagle brain (Male (L) & Female (R))

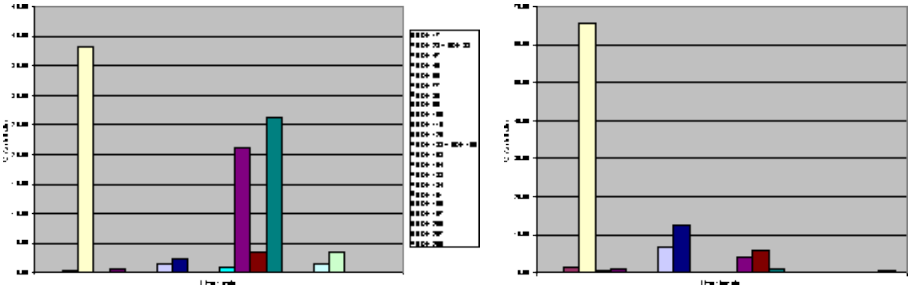

Figure 2 BDE Congener Profile White-bellied Sea-eagle fat (Male (L) & Female (R))

Figure 3 BDE Congener Profile White-bellied Sea-eagle liver (Male (L) & Female (R))

Acknowledgments

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References

[1]Müeller J., Muller R., Goudkamp K., Shaw M., Mortimer M., Haynes D., Paxman C., Hyne R., McTaggart A., Burniston D., Symons R., and Moore M. (2004) Dioxins in Aquatic Environments in Australia, National Dioxins Program Technical Report No. 6, Australian Government Department of the Environment and Heritage, Canberra, Australia (http://www.deh.gov.au/industry/chemicals/dioxins/report-6/pubs/report6a.pdf)

[2] Personal communication Dr Richard J. Montali, Veterinary Pathologist, Veterinary and Quarantine Centre, Taronga Zoo, Mosman, NSW 2088, Australia.

[3]Van den Berg, M, L. Birnbaum, A.T.C. Bosveld, B. Brunstrom, P. Cook, M. Feeley, J.P. Giesy, A. Hanberg, R. Hasegawa, S.W. Kennedy, T. Kubiak, J.C. Larsen, F.X.R. van Leeuwen, A.K.D. Liem, C. Nolt, R.E. Peterson, L. Poellinger, S. Safe, D. Schrenk, D. Tillitt, M. Tysklind, M. Younes, F. Warn, T. Zacharewski (1998) Environ. Health Persp. 106: 775-792

[4]Symons, R.K., Burniston, D., Jaber, R., Piro, N., Trout, M., Yates, A., Gales, R., Terauds, A., Pemberton D., and Robertson, D. (2003) Organohalogen Compounds 62: 257-260

[5]Symons, R.K., Burniston, D., Piro, N., Stevenson, G., Yates, A. (2004) Organohalogen Compounds 66: 3908-3914.