Polybrominated Diphenyl Ethers: Levels in Townsville sediments, Depuration and (Anti-) Estrogenic effects in Barramundi (*Lates calcarifer*)

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Declaration on Ethics

The research presented and reported in this thesis was conducted within the guidelines for research ethics outline in the *National Statement on Ethics Conduct in Research Involving Human* (1999), the *Joint NHMRC/AVCC Statement and Guidelines on Research Practice* (1997), the *James Cook University Policy on Experimentation Ethics. Standard Practices and Guidelines* (2001), and the *James Cook University Statement and Guidelines on Research Practice* (2001). The proposed research methodology received clearance from the James Cook University Experimentation Ethics Review Committee (approval number A1101).

Elisabeth Knowles Muirhead **Date**

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Abstract

The purpose of this research was to study polybrominated diphenyl ethers (PBDEs) and the effect they have in North Queensland, Australia, specifically in reference to a commercially important fish species, barramundi (*Lates calcarifer*). This thesis is separated into four main sections: determination of PBDE levels in Ross Creek, Townsville, QLD; toxicokinetics of PBDE-47 in barramundi; optimization of an enzyme-linked immunosorbent assay (ELISA) for detection of vitellogenin (Vtg) in barramundi; and assessing the (anti-)estrogenic effect of PBDE-47 in barramundi.

Levels of two common PBDE congeners, PBDE-47 and PBDE-209 were measured in sediments at three sites along Ross Creek in Townsville, QLD. Levels were found to range from below detection (0.2 μg kg^{-1} dw) to 0.35±0.2 μg kg^{-1} (dw) for PBDE-47 and from below detection (0.2 μg kg^{-1} dw) to 0.85±0.07 μg kg^{-1} (dw) for PBDE-209.

Male juvenile barramundi were injected with either a low (1 mg kg^{-1} bw) or a high (10 mg kg^{-1} bw) dose of PBDE-47 and then sampled over the course of 14 days in order to determine the depuration rate of PBDE-47 in barramundi. PBDE-47 was found to depurate at a rate of 0.041-0.069 day^{-1}, a rate which falls well within the range of the literature for depuration of PBDE-47 in fish.

An optimal ELISA for the detection of Vtg production in barramundi was determined after comparing the component reagents of a pre-existing ELISA with component reagents developed during this study. Two commercially available Vtg standards, a lipophylised Rainbow Trout Vtg standard (RT Vtg standard) and a lipophylised Atlantic Salmon Vtg standard (Salmon Vtg standard) (both from Caymen Chemical Co), were compared to a purified barramundi Vtg fraction obtained after size exclusion chromatography of plasma from barramundi in which Vtg production was induced by repeated injection of large doses of 17β-estradiol (E2). In addition, a commercially available monoclonal mouse anti-striped bass Vtg primary antibody (ND-3G2, Biosense) was compared with two polyclonal sheep anti-barramundi Vtg antibodies (Sh-0404JCU and Sh-0404-SJCU) created by inoculating sheep with one of the size exclusion

chromatography purified Vtg fractions. The optimal ELISA was determined to be the pre-existing ELISA using ND-3G2 as the primary antibody and RT Vtg standard for quantification, although promising results obtained with the purified barramundi Vtg fractions, Sh-0404JCU and Sh-0404-SJCU suggest that further purification could lead to a better barramundi specific ELISA in the future.

Finally, male, juvenile barramundi were exposed to PBDE-47 in two separate experiments to study whether PBDE-47 has an estrogenic or anti-estrogenic effect, with Vtg production measured by ELISA as the endpoint for estrogenic behaviour.

In the first experiment barramundi were given either a low (1 mg kg^{-1} bw) or a high (10 mg kg^{-1} bw) dose of PBDE-47 by intraperitoneal (i.p.) injection, and then sampled over the course of 14 days to determine the time course induction of Vtg production. Vtg levels in samples were not quantifiable but the qualitative data allowed for assessment of trends and patterns. Two interesting conclusions were apparent from the data. The first is that male barramundi appear to produce Vtg without exposure to xeno-estrogens, a hypothesis that is supported by literature that has found low natural levels of E2 production in males of many fish species. The second is that the high dose of PBDE-47 suppressed Vtg production between days 7 and 14 with Vtg levels rising much slower in the high dosed fish than in either the control or low dosed fish.

In the second experiment barramundi were given either a single low (1 mg kg^{-1} bw) or a high (10 mg kg^{-1} bw) dose of PBDE-47 by i.p. injection then sampled 3 and 6 days after injection, or were given two low (1 mg kg^{-1} bw) or a high (10 mg kg^{-1} bw) doses of PBDE-47 by i.p. injection, with three days between injections, then sampled 3 and 6 days after the second injection. This was done to determine whether a repeated dose of PBDE-47 had more of an effect on Vtg production than a single dose. The Vtg levels in these samples was quantifiable and the results showed that a double injection of PBDE-47 significantly suppressed the production of Vtg ($P<0.0001$) at both a low and high dose. In addition, at 6 days post final injection there was a small, but significant difference ($P=0.0355$) between the fish that received a single low dose and a single high dose, confirming that a single high dose of PBDE-47 can suppress Vtg production as well.

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Glossary of Abbreviations

Abbreviation	Definition
1° Ab	Primary antibody
2° Ab	Secondary antibody
ABS	Acrylonitrile Butadiene Styrene
BFR	Brominated Flame Retardant
CF	Column Fraction
DMSO	Dimethyl Sulfoxide
E2	17β-Estradiol
EDC	Endocrine Disrupting Chemical
ELISA	Enzyme-linked Immunosorbent Assay
F_{OC}	Fraction by weight of Organic Carbon
GC-MS	Gas Chromatography – Mass Spectroscopy
H+L-HRP	Horseradish Peroxidase conjugate
i.p.	Intraperitoneal
K_D	Equilibrium Constant
K_{OW}	Octanol-Water Partition Coefficient
ND-3G2	Monoclonal Mouse Anti-Striped Bass Vtg antibody (Biosense)
OPD	O-Phenylenediamine Dihydrochloride
PBDE	Polybrominated Diphenyl Ether
PBDE-47	2, 2', 4, 4'-tetrabromodiphenyl ether
PBDE-99	2, 2’, 4, 4’, 5-pentabromodiphenyl ether
PBDE-153	2, 2’, 4, 4’, 5, 5’-hexabromodiphenyl ether
PBDE-154	2, 2’, 4, 4’, 5, 6’-hexabromodiphenyl ether
PBDE-166	2, 3, 4, 4’, 5, 6-hexabromodiphenyl ether
PBDE-183	2, 2’, 3, 4, 4’, 5’, 6-heptabromodiphenyl ether
PBDE-190	2, 3, 3’, 4, 4’, 5, 6-heptabromodiphenyl ether
PBDE-205	2, 3, 3’, 4, 4’, 5, 5’, 6-octabromodiphenyl ether
PBDE-209	Decabromodiphenyl ether
PCB	Polychlorinated Biphenyl
PCB-103	2, 2’, 4, 5’, 6-pentachlorobiphenyl
PMSF	Phenylmethylsulfonyl Fluoride
POP	Persistent Organic Pollutant
RT Vtg standard	Lipophylised Rainbow Trout Vtg standard (Caymen Chemical Co)
Salmon Vtg standard	Lipophylised Atlantic Salmon Vtg standard (Caymen Chemical Co)
Sh-0404JCU	Unscreened Polyclonal Sheep Anti-Barramundi Vtg antibody
Sh-0404-SJCU	Screened Polyclonal Sheep Anti-Barramundi Vtg antibody
Vtg	Vitellogenin