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Corticosterone and Vitamin A Concentrations in Tree Swallow (Tachycineta Bicolor) Nestlings Exposed to Environmental Contaminants Along the St. Lawrence River, Canada and USA

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CORTICOSTERONE AND VITAMIN A CONCENTRATIONS IN TREE SWALLOW (TACHYCINETA BICOLOR) NESTLINGS EXPOSED TO ENVIRONMENTAL CONTAMINANTS ALONG THE ST. LAWRENCE RIVER, CANADA AND USA.

BARBARA MARTINOVIC

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Abstract

In 1999 and 2000 reproductive success and chemical biomarkers were measured in 16 day old tree swallows (*Tachycineta bicolor*) collected from nine sites along the St. Lawrence River in Canada and USA. Chicks were collected for analysis of organochlorine pesticides, polychlorinated biphenyls (PCBs), total mercury, polychlorinated dibenzodioxins (PCDDs), and polychlorinated dibenzofurans (PCDFs), and measures of corticosterone response, and vitamin A concentrations. Study sites were located in the vicinity of landfill sites and industrial outfalls in the St. Lawrence River area of concern (AOC). In 2000, the highest mean concentration of total polychlorinated biphenyls were found in chicks from Grasse River S (69.13 μg/g ww) and Grasse River N (58.64 μg/g ww). Non-ortho polychlorinated biphenyls were highest in nestlings from Turtle Creek in 2000 (0.40 μg/g ww) followed by Grasse River N (0.15 μg/g ww). In 1999, polychlorinated dibenzodioxin levels were highest at Grasse River N (79.51 ng/kg ww). The highest concentration of total polychlorinated dibenzofurans in 2000 were found in birds collected from Turtle Creek (128.50 ng/kg ww), followed by Grasse River N (120.50 ng/kg ww).

Not only did 2,3,7,8-TCDF account for the highest percentage of congener found for tetrachlorodibenzofuran (TCDF), but toxic equivalents (TEQs) 2,3,7,8-tetrachloro dibenzofuran also contributed to the highest TEQ for total dioxin and furan residues. The high concentration of contaminants in tree swallows nesting along the St. Lawrence River raise concern for bioaccumulation of lipophilic xenobiotics in the ecosystem.

There were no significant differences in reproductive parameters among sites. Corticosterone levels exhibited a diurnal rhythm in tree swallows with elevated levels in the

morning. Ten minute stress corticosterone levels were positively correlated with polychlorinated dibenzodioxin ($p=0.006$, $r=0.94$) in 1999 and 2000.

Retinol and retinyl palmitate were determined from kidney and liver samples of 16 day old tree swallow chicks. Positive correlations were found between renal molar ratio of retinol:retinyl palmitate and total tetrachlorodibenzodioxin (TCDD) ($p=0.028$, $r=0.762$).

Altered vitamin A metabolism and corticosterone homeostasis in wildlife exposed to contaminants may lead to numerous effects on reproduction, growth, and immune function. Overall these data suggest that current levels of organochlorine contamination may be affecting the hypothalamo-pituitary-adrenal axis and vitamin A levels in environmentally exposed tree swallow nestlings along the St. Lawrence River.

Dedicated to my parents, Ana and Anton Martinovic

Acknowledgements

There are many people whom I would like to thank for making this project possible. I want to take this opportunity to sincerely thank Dr. Christine Bishop for helping me to continually develop as a scientist. It is a never ending process filled with challenges and rewards, but is a wonderful and rewarding career. She has not only been a wonderful supervisor, but also a good friend who demands from her students the same hard work ethic she has herself. I would also like to thank Dr. David Lean for being my supervisor. He has also been very positive which is a much needed gift when times are trying. I have been very fortunate to have worked under these two scientists and have learned a great deal about toxicology from both of them. I would also like to thank my committee members, Dr. Pierre Mineau, Dr. Vance Trudeau and Dr. Tom Moon. They gave me numerous wonderful suggestions for improving the quality of my thesis. I would also like to extend a sincere thanks to Tom for allowing me to use his lab facilities for the corticosterone analysis. Erinn Birmingham, Glenn Barrett and Tim Sullivan are fellow tree swallow lovers who helped me greatly with this project. They spend hours on end helping monitor nests during the breeding season, and dissecting tree swallow nestlings. I must also give mention to those who provided the backbone of this project - the financial contributors and those involved in contaminant analysis. The Canadian Wildlife Service, United Fish and Wildlife Service (Anne Secord in particular), and Akwasasne Mohawk Council all financially and emotionally supported this project. I am saving the best for last. I thank my mother who is a gentle soul that always encourages me to follow my dreams. She gives me unconditional strength and love. I thank my father who has taught me the ways to aquire wisdom and persevere. I am proud of them both. I also wanted to give many thanks to my sweatie Martin Barrett. He also encourages me to follow my dreams. His own accomplishments have given me courage to persue my goals. Thanks for being my confident and love here in Ottawa.

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List of Abbreviations

PCB - polychlorinated biphenyls
PCDD - polychlorinated dibenzodioxin
PCDF - polychlorinated dibenzofuran
DDE -dichlorodiphenyldichloroethylene
Hg - mercury
TEQ - toxic equivalence
DCM - dichloromethane
GC - gas chromatograph
MS -mass spectrometer
EROD - ethoxy-o-resorufin deethylase
HPA - hypothalamo-pituitary-adrenal
R - retinol
RP - retinyl palmitate
TTR - transthyretin
RBP - retinol binding protein
LRAT - lecithin: retinol acyltransferase

"BODIES OF WATER"

We give thanks to the spirit of waters for our strength of well being. The waters of the world have provided to many: they quench thirst, provide food for plant life, and are the source of strength for many medicines we need. Once acknowledged, this too becomes a great power for those who seek its gift, for mankind himself is made from the waters.

Akwasasne Mohawk Creed

1.0 Objectives of Study

Studies of the response of wildlife and domestic species to contaminant exposure have reported increases and decreases in concentrations of corticosterone and vitamin A.

Tree swallows are being exposed to PCBs, PCDDs/PCDFs along the St. Lawrence River AOC and have exhibited lower reproductive success and depressed hepatic retinol (Bishop et al. 1999). Therefore reproductive success and vitamin A was measured in tree swallows from nine sites in the St. Lawrence River AOC to further evaluate the correlation between vitamin A and contaminant exposure. It was hypothesized that liver retinol would be depressed, and kidney retinol would be elevated in contaminated birds.

Corticosterone is an endocrine hormone which has received little attention in the past. The few field studies to date have shown that organochlorine contaminants lower plasma glucocorticoids in fish, mudpuppies and herring gull embryos (Hontela et al. 1992; Gendron et al. 1997; Lorenzen et al. 1999). Corticosterone response has not been studied in tree swallows to date. However, concentrations of total PCBs in tree swallows (5 μg/g ww) from the St. Lawrence River AOC (Bishop et al. 1999) are similar to levels of total PCBs found in wild mudpuppies and herring gull embryos exhibiting depressed corticosterone. Dioxins are also present in biota living in the St. Lawrence River AOC (Sokol et al. 1994). Laboratory studies have also shown higher corticosterone in rats exposed to TCDD (DiBartolomeis et al. 1987; Gorski et al. 1988a; 1990). Thus, plasma basal and stress level corticosterone were determined in 16 day old tree swallows nesting along the St. Lawrence River AOC. It was hypothesized that stress corticosterone would be increased in birds exposed to high levels of PCDD.

Chapter 1

1.1. LITERATURE REVIEW

1.2 Introduction

The St. Lawrence River has long been used by humans for transportation, hydroelectrical power and as a sink for human industrial wastes (Government of Canada 1991). In 1897, a canal was dug from the St. Lawrence River to the Grasse River to generate electricity. This attracted industries to the area, such as the Pittsburgh Reduction Company, which was later renamed the Aluminum Company of America (ALCOA) (Cuomo and Jorling 1990). Historically this company produced primary aluminum and fabricated wire which involved aluminum smelting, casting and fabrication. ALCOA also has numerous landfill sites which contain polychlorinated biphenyls (PCBs) and polynuclear aromatic hydrocarbons (PAHs) on their property (Cuomo and Jorling 1990). The simultaneous development of the river's transportation route and power supply became the focus of national and international deliberation in the early 1900s. Finally in 1954, Canada and the United States agreed on the development of the two projects which were accomplished by the St. Lawrence Seaway and the Moses-Saunders Power Project. Because of the availability of inexpensive electricity and shipping, large industries such as General Motors and Reynolds Metals soon came to Massena (Figure 1). General Motors was erected in 1958 in Massena, New York east of the Mohawk territory and south of Cornwall, Ontario. From 1959 to 1973 this plant used polychlorinated biphenyl (PCB) containing hydraulic fluids for die casting. Reynolds Metals Inc. is also surrounded by the St. Lawrence River, Grasse River (north), and Raquette River (south). This facility was built in 1958 to produce aluminum, and historically used a PCB based heat transfer oil. On site landfills and storage lagoons of the heat transfer fluid and oil wastes led to PCB contamination of the St. Lawrence River through leaching and wastewater discharge (Stone et al. 1991).

In 1985 the International Joint Commission (IJC) identified the St. Lawrence River near Massena, NY, as one of 42 Areas of Concern (AOC) in the Great Lakes basin where persistent toxic substances were impairing water uses (Cuomo and Jorling 1990). The St. Lawrence River AOC is approximately 80 km long, from the Moses-Saunders power dam (upstream of Cornwall) downstream to the eastern outlet of Lake St. Francis in Quebec (Drier et al. 1997).

Past studies have reported high levels of organochlorine contaminants in biota residing within the St. Lawrence River AOC (Tables 1 and 2). However, no studies have addressed the importance and impact of these high levels of organochlorine contaminants on the health of avian species breeding within the vicinity of the St. Lawrence River AOC. It was not until the early 1990s that impacts of PCBs were examined in tree swallow nestlings from the Great Lakes, including Cornwall Island and Akwasasne, the Mohawk territory (St. Lawrence River AOC). It was established that 16 day old tree swallow chicks (*Tachycineta bicolor*) accumulated up to 5.4 μg total PCBs/g of whole body tissue (ww), and that vitamin A (liver retinol) was negatively correlated with ethoxy-resorufin-*O*-deethylase (EROD) activity (Bishop et al. 1999). EROD falls under the category of mixed function oxidases (MFOs) that are a family of membrane bound enzymes which add oxygen to a wide range of

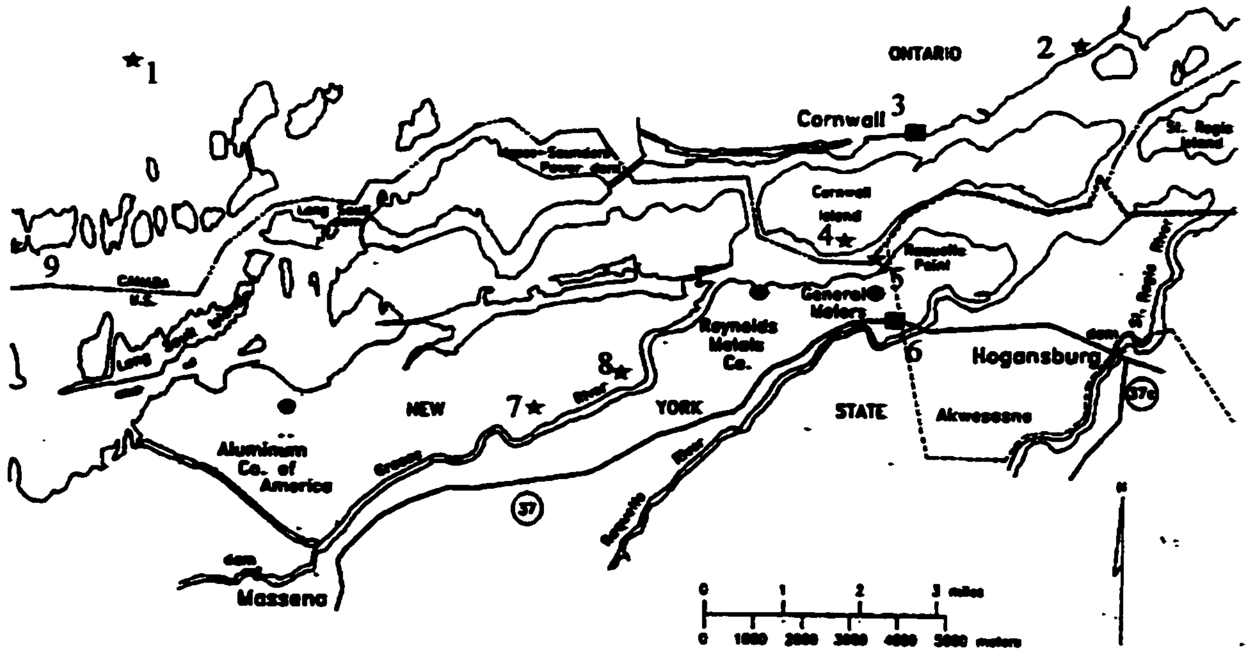


Figure 1. Map of the study site (1) Hoasic Creek; (2) Cooper Marsh; (3) Cornwall Sewage Treatment Plant; (4) Cornwall Island; (5) Turtle creek; (6) Raquette River; (7) Grasse River South; (8) Grasse River North; (9) Upper Canada Bird Sanctuary (UCBS). Water flows from Lake Ontario into the St. Lawrence River and eastward.

Site	Number	Status	lattitute/longtitude
Hoasic Creek	1	reference site	44•57'N, 57•10'W
Cooper Marsh	2	reference site	45•07'N, 74•31'W
Cornwall STP[a]	3	low PCBs AOC[b]	45•52'N, 74•42'W
Cornwall Island	4	low PCBs AOC[b]	44•59'N, 74•44'W
Turtle Creek	5	high PCBs AOC[b]	44•59'N, 74•44'W
Raquette River	6	moderate PCBs AOC	44•59'N, 74•42'W
Grasse River South	7	high PCBs AOC[b]	44•59'N, 74•38'W
Grasse River North	8	high PCBs AOC[b]	44•59'N, 74•38'W
UCBS	9	reference site	44•59'N, 75•01'W

[a] STP = Sewage Treatment Plant

[b] AOC = Area of Concern

compounds, rendering them more water soluble and thus more readily excreted. P4501A (CYP1A) is a biochemical response indicating contaminant exposure, and can be measured using the EROD assay. However, there was no direct correlation found between vitamin A and contaminant concentration in the chicks. Consequently, it is important to further evaluate the levels of vitamin A found in tree swallows nesting along the St. Lawrence River AOC. In addition, other laboratory and field studies that have examined the impacts of organochlorine contaminants on vitamin A homeostasis in birds have found both elevated and depressed levels of vitamin A, depending on biomarker tissue sampled and contaminant exposure (Spear et al. 1990; Boily et al. 1994; Murk et al. 1996; Murvoll et al. 1999; Elliott et al. 2001).

Other wildlife in the St. Lawrence River AOC and Great Lakes basin have also experienced health impacts related to environmental contaminant exposure. Mudpuppies (Amphibia) exhibited depressed corticosterone responses correlated with PCB exposure in the St. Lawrence River basin (Gendron et al. 1997). More recently, Lorenzen et al. (1999) found a negative correlation between basal corticosterone and PCDD/PCDF, total PCBs and non-ortho PCBs in herring gull (*Larus argentatus*) embryos collected from the Great Lakes. There have been no studies to date examining the effects of organochlorine contaminant exposure on corticosterone levels in tree swallow nestlings.

With the current state of the environment and rising concern over global movement of chlorinated contaminants (Bentzen et al. 1999), research on the state of animals residing in both "pristine" and "contaminated" areas is of great importance. Links between contaminants and the endocrine system (corticosterone), as well as modulators of growth and reproduction (vitamin A), need to be established in order to understand the potential effects of xenobiotics on wildlife species. By examining the impacts of contaminant exposure on Vitamin A and corticosterone in tree swallows nesting in the St. Lawrence River AOC, we may begin to address the complexity of health impacts that may be induced by chlorinated hydrocarbons in wild bird populations.

1.1.2. The tree swallow as a sentinel species

The tree swallow is a common breeding passerine throughout the northern half of North America, where it is frequently found in association with bodies of water. As an aerial insectivore, the tree swallow feeds primarily on flying insects during the breeding season, in close proximity to its nest (within 2 km) (Robertson et al. 1992). It is a cavity nester which cannot excavate its own cavitiy. Thus they can easily be induced to nest in colonies of artificial nest-boxes. Its use of nest-boxes for breeding, combined with its tendency to feed on insects emerging from bodies of water located near the nest site, make the tree swallow an excellent candidate for the monitoring of aquatic contaminants (Hebert et al. 1993; Secord and McCarty 1997). Nestlings are known to bioaccumulate methylmercury (110 ng/g dw) (Gerrard and St. Louis 2001), dioxins (2.4 ng/kg) (Ankley et al. 1993), and PCBs (5.4 μg/g) (Bishop et al. 1999) from contaminated wetlands.

The following review of relevant research provides a summary of the effects of organochlorine contaminants on corticosterone and vitamin A levels as they relate to animals exposed in the field and in the laboratory.

Table 1. Contaminant levels in wildlife collected from the St. Lawrence River Area of Concern.

SPECIES (SEX)	SITE	TISSUE	MASS (G) OF ANIMAL	PCB[A] (µg/g)	TCDD[B] (ng/g)*	TCDF[C] (ng/g)*	REF
Bullfrog (M)	Raquette R	Muscle	159	ND	NA	NA	Stone et al. 1991
Bullfrog (M)	Grasse R	Muscle	116	0.2	1.7	1.8	Stone et al. 1991
Bullfrog (F)	Turtle C	Muscle	282	7.2	1.2	7.7	Stone et al. 1991
Snapping Turtle (M)	Turtle C	Muscle	11200	2.7	NA	NA	Stone et al. 1991
Snapping Turtle (M)	Turtle C	Liver	11200	62.0	NA	NA	Stone et al. 1991
Snapping Turtle (M)	Grasse R	Liver	7300	94.6	340	22	Stone et al. 1991
Snapping Turtle (M)	Raquette R	Liver	6400	2.8	NA	NA	Stone et al. 1991
Muskrat (F)	Raquette R Turtle C	Liver	NA	ND	NA	NA	Stone et al. 1991
Mallard[D] (M)	Turtle C	Muscle	NA	349	13	1990	Stone et al. 1991
Mallard[D] (M)	Raquette R	Muscle	NA	ND	5.3	120	Stone et al. 1991
Common Merganser[E] (F)	Grasse R (near Reynold Metals)	Muscle	NA	70	76	460	Stone et al. 1991
Common Merganser[E] (M)	Turtle C	Muscle	NA	240	NA	NA	Stone et al. 1991
Tree swallow (16 d old)	Turtle C	whole body	19-21	5.4	NA	NA	Bishop et al. 1999
	Cornwall Island		19-21	1.4	NA	NA	

[A] Total PCBs = sum of PCB 1248 and PCB 1260
[B] TCDD = sum of 2,3,7,8-TCDD, 1,2,3,7,8-PeCDD, 1,2,3,6,7,8-HxCDD, 1,2,3,4,6,7,8-HpCDD, 1,2,3,4,5,,7,8,9-OCDD
[C] TCDF = sum of 2,3,7,8-TCDF, 1,2,3,7,8-PeCDF, 1,2,3,6,7,8-HxCDF, 1,2,3,4,6,7,8-HpCDF, 1,2,3,4,5,,7,8,9-OCDF

[D] AHY age, after hatch year (bird collected in a calendar year after that in which it was hatched).

[E] HY age, hatch year (bird collected in the same calendar year in which it hatched)

ND = Not Detected; wet weight detection limit for PCBs is 0.1 µg/g.

- = All dioxin and furan samples measured in fat tissue of all animals excluding frogs (measured in muscle).

All animal tissue collected in 1988/1989, except study by Bishop et al (1999) collected in 1994.

Table 2. Contaminant residues (µg/g) in Young-of-the Year Spottail Shiners (Suns et al. 1991).

SITE	YEAR	TOTAL FISH LENGTH (mm)	Total PCBs
Cornwall Island (S)	1989	46	0.39
Grasse River	1979	51	2.07
Grasse River	1989	50	0.91
Turtle Creek	1987	50	1.26
Turtle Creek	1989	50	0.02
Raquette River	1979	50	0.38
Raquette River	1989	36	1.20
Reynolds Metals	1989	53	16.60

Note: Values are means in µg/g ww of whole fish analysis.

1.1.3 PCBs (Polychlorinated biphenyls), PCDDs (Polychlorinated dibenzodioxin) and PCDFs (Polychlorinated dibenzofuran)

PCBs have been in general use since 1930, having appeared in commercial products (Aroclor mixtures) as heat transfer agents, lubricants, dielectric agents, flame retardants and plasticizers (Safe 1994). However, their predominant use has been as an insulating and cooling agent in electrical transformers. This is critical because there are a number of industries along the St. Lawrence River and its tributaries which historically used PCBs and discharged their industrial wastewater into this river system (Stone et al. 1991).

Properties which made PCBs appealing for industrial use, also continue to make PCBs a contaminant of concern (Figure 2). Low water solubility, resistance to chemical breakdown, and the ability to bioaccumulate in the food chain have led to accumulation of PCBs in birds residing within the St. Lawrence River AOC (Bishop et al. 1999). Because PCBs are hydrophobic, they are taken up by fatty tissues of benthic invertebrates which spend a portion of their life cycle in close proximity to sediments which adhere PCB molecules to their carbon atoms (Froese et al. 1998). This process continues as insectivorous birds accumulate higher concentrations (biomagnify) of these chemicals as they become deposited in the fat of the body.

Similar to PCBs, polychlorinated dibenzodioxin (PCDD) and polychlorinated dibenzo-o-furan (PCDF) (Figure 3) are detected in almost every component of the global environment (Environment Canada 1998). These compounds were not intentionally produced but are formed as byproducts of numerous industrial processes

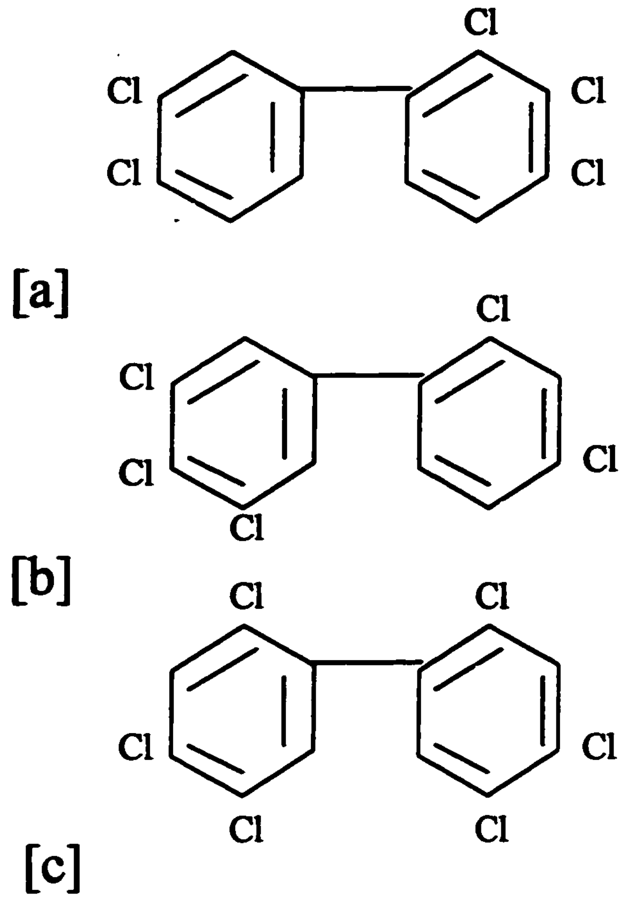
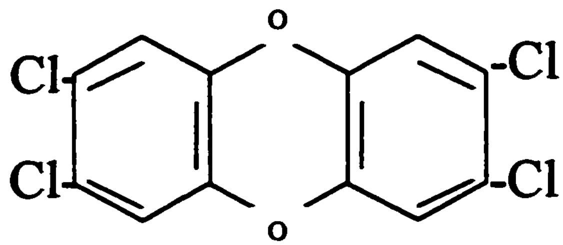
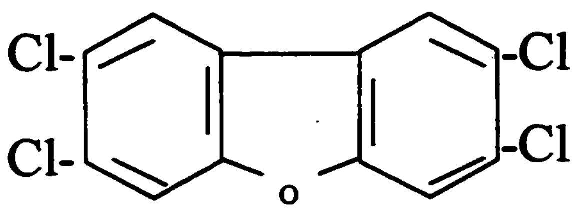


Figure 2. Structure of polychlorinated biphenyls. [A] PCB 105 (2,3,3',4,4' pentachlorobiphenyl); [B] PCB 118 (2,3',4,4',5 pentachlorobiphenyl) and [C] PCB 153 (2,2',4,4',5,5' hexachlorobiphenyl). Ammended from Safe 1994.



[a]



[b]

Figure 3. Structure of [A] 2,3,7,8 tetrachlorinated dibenzo-dioxin and [B] 2,3,7,8 tetrachlorinated dibenzo-furan. Ammended from Safe 1994.

including the synthesis of diverse chlorinated aromatics, production and smelting of metallic ores, pulp and paper production, and combustion of municipal and industrial wastes (Keul et al. 1989; Environment Canada 1998). Polychlorinated dibenzodioxin and polychlorinated dibenzofurans are two related classes of aromatic compounds which also have low water solubility, tend to adhere to fatty tissue and have been documented in birds collected from the shores of the St. Lawrence River AOC (Stone et al. 1991). The most toxic of these is 2,3,7,8-tetrachlorinated dibenzodioxin, with chlorine atoms substituted on the four lateral positions of the molecule (Figure 3) (Safe 1994).

Toxic effects in birds exposed to PCB mixtures and/or PCDD/PCDF have included wasting syndrome, thymic atrophy, altered vitamin A levels, reproductive impairment, and lower corticosterone levels (Cecil et al. 1973; Brouwer and van den Berg 1986; Gilbertson et al. 1991; Fox 1993; Secord and McCarty 1997; Lorenzen et al. 1999; Elliott et al. 2001). Structure-toxicity relationships support a role for the interaction between TetraCDD and related PCDD, PCDF, non-ortho substituted PCBs and altered endogenous enzyme systems important for normal homeostasis of biological systems (Environment Canada 1998). The coplanar shape with chlorine atoms substituted on the meta and para positions of the molecule plays an important role for binding to the aryl hydrocarbon-receptor. The chemical inducer binds to the cytosolic aryl hydrocarbon-receptor to form a complex which binds to the dioxin responsive element to enhance gene expression of CYP1A1. CYP1A1 (P4501A) is part of the mixed function oxidase (MFO) system responsible for adding oxygen to compounds such as PCBs to render them more water soluble and excretable by the body. Changes in these enzymes may result in secondary effects or changes in steroid homeostasis, and vitamin A production (Fox

1993). Of particular concern are non-ortho PCB congeners 3,3',4,4' tetraCB (PCB 77), 3,3',4,4',5 penta CB (PCB 126), and 3,3',4,4',5,5' hexaCB (PCB 169) (Figure 4). They can induce aryl hydrocarbon hydroxylase activity and bind to the cytosol receptor, as does 2,3,7,8-tetrachlordibenzo-p-dioxin (TCDD), because of their coplanar shape with chlorine atoms substituted at the meta and para positions of the molecule (Hansen 1987). However, the non-ortho PCBs exhibit lower receptor affinity (100 times less) and biological potency as compared to TCDD (Poland and Knutson 1982).

The TEQ (toxic equivalency) approach has been used in risk assessment of PCDD/PCDF and PCBs for mammals and birds, and is based on structure and affinity of the congener for the cytosolic receptor. Congeners have been assigned a toxic equivalency factor (TEF), which is the fractional toxicity of the congener relative to a standard toxin, i.e. 2,3,7,8-TCDD (Ahlborg et al. 1992). TEF values can be derived by exposing cell cultures [like the H4IIE rat hepatoma culture (Safe 1994), or chicken embryo hepatocytes (Kennedy et al. 1996)] to a sample extract and measure the induction of enzymes known to respond to chlorinated hydrocarbons.

1.1.4 Organochlorine contaminants and corticosterone

Corticosterone is an adrenal hormone (Figure 5) involved in protein and carbohydrate metabolism through a process called gluconeogenesis (Norris 1997). An extensive literature review by Ribelin (1984) revealed that the adrenal gland, responsible for corticosterone release, suffered more structural lesions from experimental and clinical chemicals than other endrocrine organs (including testis, thyroid, ovary, pancreas, pituitary, and parathyroid). The vulnerability of the adrenal gland results from its fatty composition, and high vascularization.

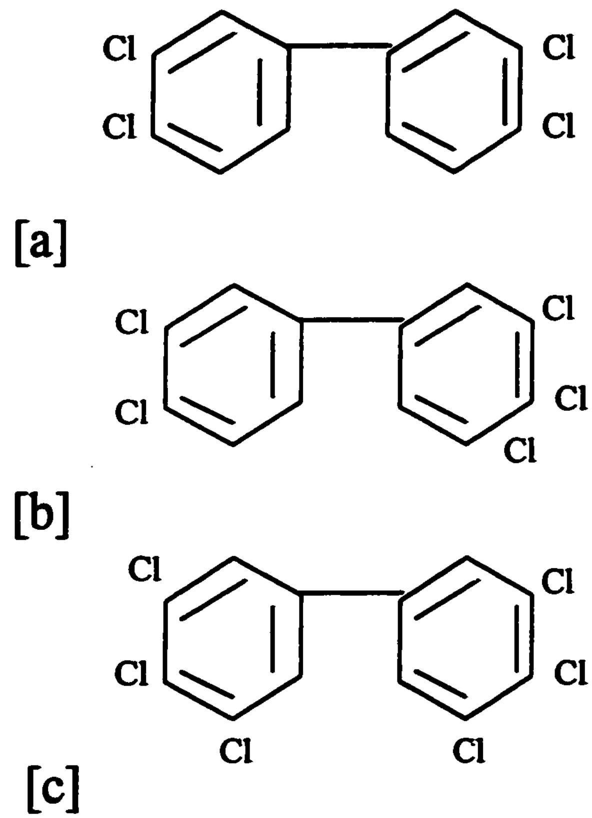


Figure 4. Structure of non-ortho polychlorinated biphenyls [A] PCB 77 (3,3',4,4' tetrachlorobiphenyl); [B] PCB 126 (3,3',4,4',5 pentachlorobiphenyl); and [C] PCB 169 (3,3',4,4',5,5' hexachlorobiphenyl). Ammended from Safe 1994.

lipophilic contaminants that enter the body are therefore deposited in the lipid rich adrenal gland.

In addition, numerous other sites along the hypothalamo-pituitary-adrenal (HPA) axis may also be susceptible to contaminants (Figure 6), ultimately impacting corticosterone production. Although the vulnerability of corticosterone secretion is apparent, few studies have been devoted to changes in the hypothalamo-pituitary-adrenal axis in birds exposed to organochlorine contaminants (Table 3).

Field Studies

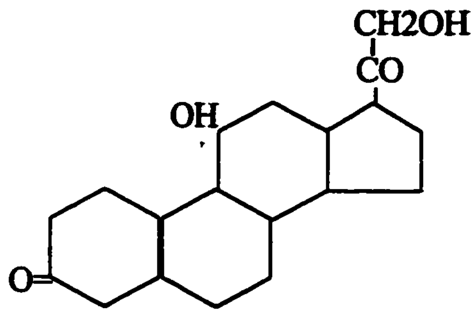
Herring gulls

Lorenzen et al. (1999) examined the correlation among environmental organochlorine contaminant residues and plasma corticosterone levels in Great Lakes herring gull embryos. Regression analysis of the sum of individual yolk sac PCDD/PCDF, total PCBs, and non-ortho PCBs with corticosterone concentrations indicated inverse relationships, suggesting that environmental levels of contaminants were sufficient to disrupt the HPA axis in herring gull embryos.

Amphibians and Fish

The exposure route of organochlorine contaminants for some birds is quite different from amphibians and fish. Amphibians are mainly herbivores as tadpoles and insectivorous as adults, and the complex life cycles of some species of Anuran involve both aquatic and terrestrial phases, potentially exposing them to different xenobiotics (Bishop and Martinovic 2000). Fish can be exposed to pollutants through their thin and richly vascularized gills, through skin and by consuming contaminated food (Hontela

Corticosterone



Cortisol

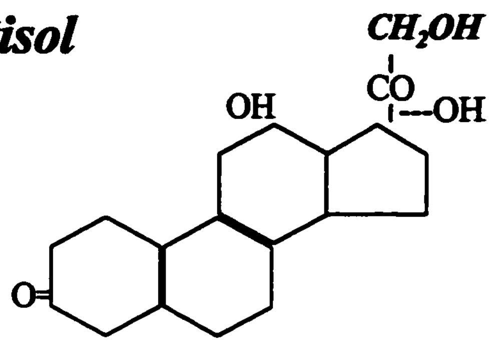


Figure 5. Structure of corticosterone and cortisol. Ammended from Norris 1997.

Hypothalamo-Pituitary-Adrenal axis

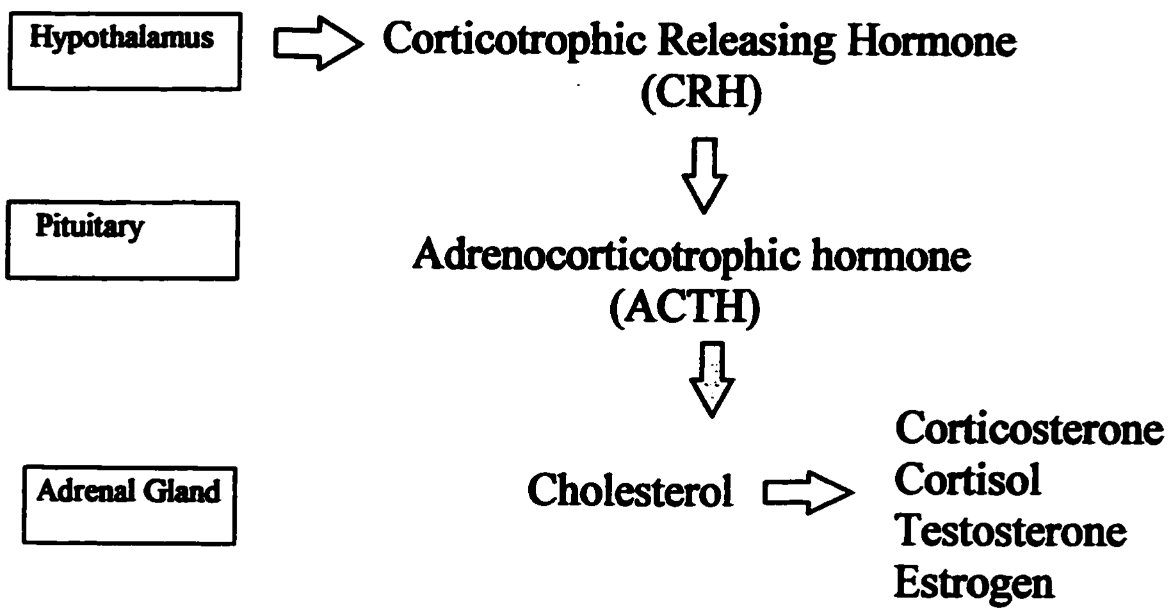


Figure 5. Cellular processes within the hypothalamo-pituitary-adrenal axis (HPA). Release of corticotrophic releasing hormone from hypothalamus to initiate release of adrenocortocotrophic (ACTH) hormone from the pituitary which targets the adrenal gland. Cholesterol is converted into endocrine hormones including corticosterone (released by adrenal gland). Ammended from Norris 1997.

Table 3. Field and laboratory studies on effects of organochlorine contaminants, polynuclear aromatic hydrocarbons (PAHs) and total mercury (Hg) on plasma corticosterone.

Chemical	Species	Highest mean concentration in tissue* & biomarker effect	Reference
Total PCBs	mudpuppy	decrease corticosterone	Gendron et al. 1997
PAHs, PCBs, Hg (total)	yellow perch	- decrease cortisol	Hontela et al. 1992; 1995
PCDD/ PCDF	herring gull embryo - field	< .001 µg/g ww- Decrease basal corticosterone levels	Lorenzen et al. 1999
total PCBs	herring gull	PCBs - decrease corticosterone	Lorenzen et al. 1999
Non-ortho PCBs	herring gull	0.01 µg/g - decrease corticosterone	Lorenzen et al. 1999
Aroclor 1254	rat	0.02-.12 µg/g - elevates basal corticosterone, not stress induced corticosterone	Miller et al. 1993
TCDD	rat	0.50 µg/g - increase corticosterone in AM	DiBartolomeis et al. 1987
TCDD	rat	0.12 µg/g - decrease gluconeogenesis	Gorski et al. 1990
TCDD	rat	0.12 µg/g - increase corticosterone	Gorski et al. 1988a
TCDD	rat	0.12 µg/g - corticosterone decrease toxicity of TCDD	Gorski et al. 1988b

TCDD - tetrachlorodibenzo dioxin
PEPCK - phosphoenolpyruvate carboxykinase
PAH - polynuclear aromatic hydrocarbon
Note: In field studies highest mean concentration found on ww basis; in laboratory studies highest concentration administered

1997). In contrast, birds are sensitive to bioaccumulation of lipophilic contaminants only from food sources which inhabit these contaminated water bodies. However, all three classes of vertebrates have exhibited reduced corticosterone concentrations when collected from contaminated wetlands.

Gendron et al. (1997) sampled mudpuppies (*Necturis maculosis*) from the St. Lawrence and Ottawa Rivers for contaminant levels (measured in the gonads) and integrity of the hypothalamo-pituitary-interrenal (HPI) axis. Mudpuppies subjected to an *in situ* ACTH challenge 24 h after capture had lower surges in corticosterone, implying the disruption of the HPI axis downstream of the pituitary. It was also noted that recovery levels of resting corticosterone were significantly higher in animals from contaminated sites. They proposed that there could be a decrease in the clearance rate or a change in the regulatory feedback loop of corticosterone. Normally, high levels of corticosterone can cause a decrease in the release of adrenocorticotrophic hormone (ACTH) from the pituitary (Norris 1997).

Yellow perch (*Perca flavecsens*) and northern pike (*Esox lucius*) collected from sites contaminated with mixtures of polyaromatic nuclear hydrocarbons (PAHs), PCBs, and heavy metals exhibited a reduced capacity to elevate plasma cortisol in response to a standardized capture stress (Hontela et al. 1992; 1995). *In vivo* challenges involving ACTH administration were also conducted on yellow perch from contaminated sites. Cortisol impairment could not be reversed with ACTH replacement, indicating that an interrenal rather than a pituitary dysfunction is responsible for the reduced capacity to secrete cortisol.

Laboratory studies

Although few field studies have examined corticosterone and cortisol in animals exposed to organochlorine contaminants, many laboratory studies have found effects of PCBs and PCDDs on corticosterone levels. In addition, laboratory studies are better able to control for confounding factors. Temperature and weather changes, food abundance, and age of the animals are factors that impact corticosterone (Wingfield et al. 1984; Norris 1997). The following laboratory studies have been included to aid in the understanding of impacts of organochlorine contaminants on corticosterone concentrations.

Rodent studies

Effects of PCBs and dioxins on adrenal function in rats and mice have been investigated in several studies. However the cause and effect relationship between contaminants and plasma corticosterone is still unclear. Similar to results from field studies, laboratory mice and rats exposed to dietary organochlorines have also shown depressed levels of corticosterone. For example, adult rats fed 50 μg/g PCBs (Aroclor 1254, 1242, 1016) had decreased plasma corticosterone levels and decreased adrenal weights (Byrne et al. 1988). Even groups of rats fed 1, 5 and 10 μg/g Aroclor 1254 showed a 50% reduction in corticosterone levels compared with the control group. Young rats born to mothers fed PCBs had an impaired ability to elevate corticosterone in response to adrenocorticotropic hormone (ACTH), although without stimulation, the plasma corticosterone levels were not different between control rats and rats exposed to PCBs (Meserve et al. 1992).

Corticosterone and ACTH concentrations were measured in rats treated with a single oral dose of TCDD (Bestervelt et al. 1993). Although ACTH levels were higher during the exposure to TCDD, corticosterone levels were suppressed significantly by day 14 in primary cultures of rat adrenal cells. The authors proposed that TCDD decreased the bioactivity of the ACTH secreted by the pituitary (Bestervelt et al. 1993). ACTH is known to exist in several different processed and unprocessed forms with varying steroidogenic potencies. ACTH is synthesized from a large precursor molecule (31KD) which is cleaved to a 22KD intermediate, and further cleaved to yield a 16KD and 4.5KD ACTH. The 4.5KD ACTH is the most bioactive as measured by the synthesis and secretion of corticosterone by rat adrenal cell cultures. Therefore, the authors proposed that production of the 31KD and 22KD peptides would produce less active ACTH which is less able to stimulate release of corticosterone.

Reports of elevated levels of corticostorone are also evident in the literature. Plasma corticosterone in rats fed a single dose of TCDD rose five to seven times higher than the control group (Gorski et al. 1988a). In addition, morphological changes were seen in the adrenal gland as characterised by an increase in size of the zona fasciculata where corticosterone is secreted. The authors proposed that TCDD was exerting effects on the adrenal gland to produce more corticosterone.

Miller et al. (1993) exposed weanling rats to Aroclor 1254 for 5, 19, and 15 weeks on a daily basis. By week 15, all dosing groups displayed increased corticosterone. However, no changes in adrenal weight accompanied the corticosterone change, indicating that the effect of Aroclor 1254 may be acting indirectly on corticosterone.

1.1.5 The corticosterone hypothesis

One way to think about many glucocorticoid responses has been to consider them as preparations for stressful situation (Wingfield 1999). This statement has been the basis for recent work on corticosterone in both field and laboratory studies. In testing for integrity of the hypothalamo-pituitary-adrenal (HPA) axis, researchers can take blood samples to analyse for "resting" or basal levels of corticosterone and also expose the test organism to standardized stress tests, or inject ACTH to evaluate the response of plasma corticosterone. However, corticosterone levels often increase within minutes of animal capture, leading to more confounding variables in field experiments. For example, Gendron et al. (1997) sampled mudpuppies from the St. Lawrence and Ottawa Rivers and found depressed basal corticosterone concentrations. Upon injection of ACTH (the hormone involved in elevating corticosterone), mudpuppies failed to increase corticosterone. The authors proposed that there was a disruption in the HPI axis downstream of ACTH release from the pituitary. However, mudpuppies were captured in traps for unknown and inconsistent lengths of time. It is plausable that corticosterone levels were unable to increase because corticosterone had reached their maximal stress levels. Field and laboratory studies have overcome this problem by acclimating the animal, and allowing time for corticosterone to return to basal levels (DiBartolomeis et al. 1987; Gorski et al. 1988; Hontela et al. 1992; 1995). Similarly, blood sampling birds within one minute of capture gives measurement of basal corticosterone (Wingfield et al. 1984; Westerhof and Lumeij 1996).

When comparing field studies, seasonal and diurnal rhythms must be taken into account. Seasonal and diurnal rhythms of corticosterone have been found in a number of species including fish (Hontela 1997), amphibians (Leboulenger et al. 1982) and birds (Wingfield 1984; Westerhof et al. 1994). During migration, gray catbirds (*Dumetella carolinensis*) have exhibited an inability to raise corticosterone after exposure to handling stress flight (Holberton et al. 1996). Since corticosterone utilizes amino acid sources from protein for carbohydrate metabolism, corticosterone is suppressed to protect muscle needed for flight. Consequently avian studies in particular should consider the season for field sampling, time of day, and the age of the animal.

Environmental contaminants may also influence the diurnal rhythm of steroid hormones. Some lab and field studies neglect to include time of sampling in their methodology. DiBartolomeis et al. (1987) found an interesting relationship between diurnal rhythms in corticosterone and TCDD. Rats exposed to a single treatment of 2,3,7,8-tetrachlorodibenzo dioxin (TCDD) demonstrated higher levels of corticosterone in the morning and a decrease of corticosterone by 40% in the evening, compared with control rats. When rats were given lower doses of TCDD, there was no increase in corticosterone in the morning. However, corticosterone was still depressed by 40% in rats sampled in the evening. The authors concluded that TCDD may have caused an increase in cholesterol transfer, where as the evening decrease in corticosterone may have been the result of TCDD inhibition of cholesterol side chain cleavage. This inhibition may be the result of the side-chain cleavage becoming more limiting for corticosterone synthesis when cholesterol transfer is higher, as for PM peak secretion. These daily fluctuations may explain some of the differences observed between increased and decreased levels of corticosterone in field and laboratory studies.

Plasma corticosterone is known to increase during fasting or starvation as part of a multihormonal response to stimulate gluconeogenesis (Simon 1984), which is needed to maintain glucose homeostasis (Blomhoff 1994). Consequently diet and body condition are important factors which affect corticosterone levels. It is especially critical when researching the correlation between TCDD and corticosterone, that body condition (body weight and lipid) is taken into account.

Lethal doses of TCDD have caused profound reductions in feed intake in laboratory rats (Gorski et al. 1988) leading to the postulation that elevations in plasma were due to starvation stress. However, rats exposed to sublethal levels of TCDD experienced the same magnitude of elevation in corticosterone compared with control groups, and also displayed changes in adrenal morphology. The authors concluded that the observed increase in corticosterone was not a consequence of starvation alone. These results add to the importance of sampling time, since morning and afternoon sampling may be different as a result of "natural" rhythms and/or contaminant exposure.

Although lab studies can monitor food intake with relative ease, field studies are more difficult. Hence body weight, stomach content of food, and fat scores are indices which can be incorporated into field studies. For example, Gendron et al. (1997) concluded from stomach content that all the mudpuppies sampled were feeding on the same bait, with similar stomach quantities between sites, and was not correlated with depressed corticosterone levels.

The consequences of an impairment on the physiological performance remain to be assessed. It can be postulated however, that depressed levels of corticosterone may diminish an animal's ability to respond to stressors (fight or flight). In addition, chronic elevated levels of corticosterone may result in enhanced mobilization of protein for gluconeogenesis, resulting in lower muscle mass and compromised flight.

1.1.6 Organochlorine contaminants and Vitamin A

Vitamin A is a fat soluble essential vitamin (Figure 8) involved in growth, reproduction, vision, and cellular immune function (Fox 1993). Several studies have shown that birds display altered levels of vitamin A (both retinol and retinyl palmitate) in plasma and liver when exposed to PCBs and PCDD/PCDF (Spear et al. 1986; 1989; 1990; Boily et al. 1994; Bishop et al. 1999; Murvoll et al. 1999; Elliott et al. 2001). Since PCBs and dioxins are still found in the aquatic environment and have potential to bioaccumulate through the food chain, additional research on the impacts of these organochlorine contaminants on vitamin A concentrations of birds is crucial. A review of recent findings concerning PCBs, PCDD/PCDF exposure and avian levels of vitamin A is presented in Table 4.

Field Studies

Field studies are extremely important as they provide an indication of the vulnerability of vitamin A homeostasis in avian species with respect to PCB and PCDD/PCDF exposure in their natural habitat. Studies of this nature are more complex than laboratory studies as variables such as dietary carotene and retinol, weather, and condition of the animal must be taken into account.

One of the first field studies to document a decrease in retinoid levels in herring gulls was conducted in 1982 from three Great Lakes sites and an Atlantic coast colony (Kent island, New Brunswick) (Spear et al. 1986). Within the Great Lakes, levels of liver retinol were lowest in Lake Ontario and highest in Lake Superior gulls. In addition, liver retinol showed a statistically significant relationship to arylhydrocarbon hydroxylase (AHH) activity, indicative of contaminant exposure (Fox 1993). Although there were no chemical analysis carried out on these specific eggs, low levels of vitamin A paralleled high levels of TCDD found in herring gull eggs collected in these areas in 1980 and 1983.

A more intensive study conducted by Environment Canada between 1980-1985 found that liver retinol and retinyl palmitate varied widely in adult herring gulls from the Atlantic coast colonies, both between locations and between years, but that levels were generally higher in the Atlantic coast colonies compared to the 11 Great Lakes colonies (Government of Canada 1991).

A more recent study of tree swallow nestlings from the St. Lawrence River found no association between PCBs and hepatic retinoid levels (Bishop et al. 1999). However, liver ethoxy-resorufin deethylase (EROD) activity was negatively correlated with liver retinol levels. EROD activity is a sensitive biomarker of exposure to TCDD and other compounds which act on the Aryl hydrocarbon receptor (Ah) (Kubiak et al. 1989). This leads to the conclusion that exposure to environmental contaminants is affecting vitamin A levels in tree swallow chicks. Dietary intake of β-carotene was found to be sufficient in the nestlings indicating no limited availability of vitamin A.

Great blue heron eggs collected from nine heronries from the St. Lawrence River and two reference sites were analyzed for different forms of vitamin A. Retinyl palmitate in Great blue heron egg yolk was negatively correlated with PCB 105 and 118 (wet weight) (Boily et al. 1994). The authors also examined retinol and β–carotene in heron eggs, but did not find significant diferences between colonies.

Table 4. Avian field studies on effects of Polychlorinated biphenyls (PCBs) and Dioxins on vitamin A concentrations.

Chemical	Species	Tissues sampled	Effect	Reference
PCB 118 + 105	GBH-field	egg yolk	7.8 μg/g ww- retinyl palmitate negatively correlated with both congeners	Boily et al. 1994
2,3,7,8-TCDD	herring gulls-field	egg yolk	0.06 ng/g ww- decreased retinol	Spear et al. 1986
2,3,7,8-TCDD	herring gull eggs - field	egg yolk	0.13 ng/g ww - increase molar ratio of retinol:retinyl palmitate	Spear et al. 1990
total PCBs	16 d tree swallow-field	liver & kidney	5μg/g ww EROD activity is negatively correlated with hepatic retinol	Bishop et al. 1999
total PCBs	Tern chicks-field	plasma	EROD activity is positively correlated with plasma retinol	Murk et al. 1996
total PCBs	Osprey chicks - field	liver & kidney	Vitamin A positively correlated with PCBs	Elliott et al. 2001
total PCBs	Shag - field	plasma	1.22 μg/g ww or 18 μg/g lw eggs - increase in retinol	Murvoll et al. 1999

TCB - tetrachlorobiphenyl
TCDD - tetrachlorodibenzo dioxin
PCBs - polychlorinated biphenyls
GBH - Great Blue Heron
ww - weight wet; lw - lipid weight

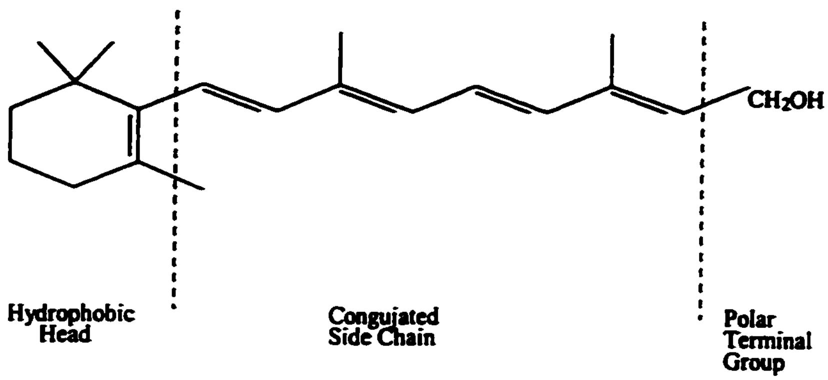


Figure 7. Structure of retinol, the circulating form of vitamin A.

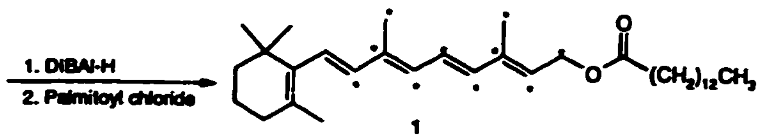


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Figure 8. Structure of retinyl palmitate, the storage form of vitamin A.

To evaluate the possibility of applying plasma retinol as a biomarker of response in seabirds exposed to chronic low levels of organochlorines, the relationship between yolk content of PCBs and plasma retinol levels were studied in newly hatched shag chicks (*Plalacrocorax aristotelis*) from the coast of central Norway (Murvoll et al. 1999). The study concluded that plasma retinol (lipid weight) and organochlorine exposure to newly hatched shag chicks were positively correlated.

Spear et al. (1990) found that the molar ratio of retinol to retinyl palmitate (in egg yolk) was positively correlated with the sum of TCDD and TCDF concentration, and with 2,3,7,8-TCDD in herring gull eggs collected during early incubation from seven colonies around the Great Lakes. However, no correlation was found between contaminant levels and retinol or retinyl palmitate individually. The authors also found that embryonic age affected retinoid content. Earlier embryonic stages had higher levels of retinol, presumably due to a lack of enzymes involved in converting retinol to retinyl palmitate.

Osprey (*Pandion haliaetus*) eggs were collected along the Fraser and Columbia River systems of British Columbia, Washington and Oregon and analysed for total PCBs and TCDDs. Of the eggs collected, thirty eight were hatched in a laboratory incubator and sacrificed within 24 for biomarker analysis (Elliott et al. 2001). Osprey chicks exhibited a positive correlation between liver retinol and yolk sac PCBs.

1.1.7 The Vitamin A hypotheses

A number of investigations have reported lower levels of vitamin A in birds exposed to organochlorine contaminants (Spear et al. 1986; 1989; Government of Canada 1991; Boily et al. 1994; Bishop et al. 1999), while other studies have demonstrated a positive correlation between vitamin A concentrations in birds and PCBs and/or TCDD concentrations (Spear et al. 1990; Murvoll et al. 1999; Elliott et al. 2001).

The tissue analysed for retinoids must be addressed since this may impact interpretation of results. A number of studies have found depressed concentrations of hepatic retinol in nestling and adult birds environmentally exposed to organochlorine contaminants (Spear et al. 1986; Government of Canada 1991; Bishop et al. 1999). The metabolic pathway of vitamin A is complex and involves distribution through a number of target tissues (Figure 9). However over 95% of vitamin A is found in the liver as retinyl palmitate, which can be hydrolyzed to retinol, depending on dietary intake and physiological requirements (Zile 1992). It has been proposed that PCBs, PCDDs, and PCDFs can bind to the aryl hydrocarbon receptor where it can transcribe specific proteins, some of which regulate vitamin A storage and catabolism (Zile 1992). Although the liver is an important biomarker tissue in determining impacts of organochlorine contaminants on vitamin A, the kidney also has an important role in vitamin A metabolism. Bishop et al. (1999) found a negative trend between kidney retinol and EROD activity in tree swallow chicks. Elliott et al. (2001) found differences among sites for kidney retinoids. However, there were no correlations between kidney vitamin A and contaminant concentrations in osprey chicks. Since the kidney is an organ of filtration, any retinol displaced from its carrier protein will be in part transported to the kidney. It may be possible that decreased enzyme activity involved in metabolic breakdown of retinol for filtration through the kidney. The role for cellular retinol binding protein (CRBP) may also influence kidney vitamin A (Levin and Davis 1997). Increased levels of RBP in the kidney may result in increase uptake of retinol from serum, and decrease retinyl palmitate via its effects on the lecithin:retinol acyltransferase (LRAT), responsible for esterication of retinol.

Murvoll et al. (1999) found a borderline positive correlation (p=0.05) between total PCBs (lipid weight) and plasma retinol in shag chicks. Murk et al. (1996) found a positive relationship betwen EROD activity and plasma retinol in tern chicks. Some organochlorine hydroxy metabolites are structurally similar to thyroxine and have a high affinity for its binding site on transthyretin (TTR). Hydroxy metabolites that displace thyroxine induce conformational change in TTR, causing a dissociation of the retinol binding protein (RBP)-retinol complex (Brouwer et al. 1986), which is then more readily filtered by the kidney (Green and Green 1994). Although plasma retinol has been used as a biomarker of toxicity exposure, the liver is a major target organ for vitamin A activity and thus may provide a more sensitive organ for detecting changes in vitamin A homeostasis.

When making a comparison between field studies it is also important to note that several studies did not measure dietary vitamin A (Spear et al. 1986; Government of Canada 1991; Murvoll et al. 1999; Elliott et al. 2001). Because retinoid concentrations tend to reflect vitamin A availability in the diet, it is critical to establish levels of retinoid precursors such as β–carotene to account for dietary influence.

Bishop et al. (1999) analysed insect boluses (aerial insects collected by parents and fed to growing chicks) from eight to ten day old tree swallows nesting along the shores of the Great Lakes and St. Lawrence River. Although no differences were found between sites for β–carotene, liver retinol was negatively correlated with EROD activity. The

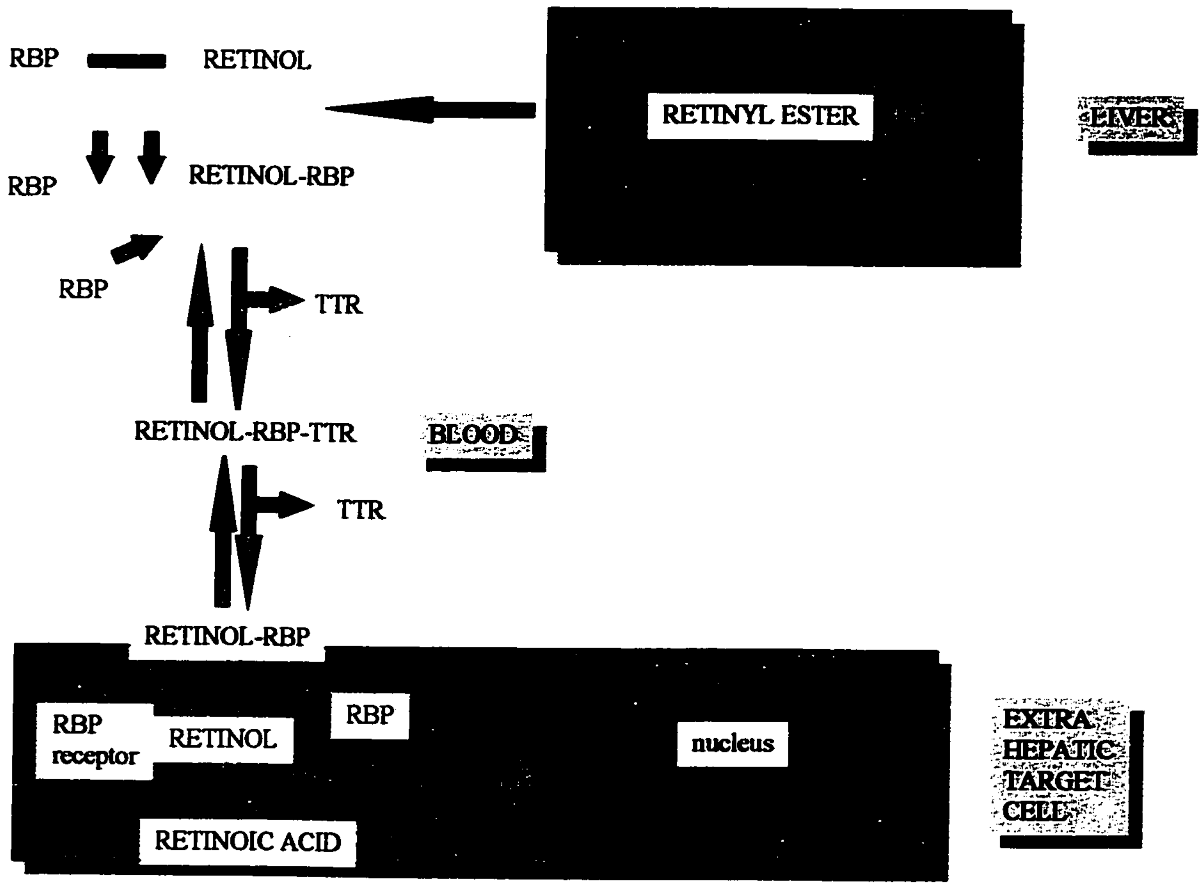


Figure 9. Vitamin A pathway. Retinyl palmitate ester (RE) is converted to retinol (R). Retinol is bound to retinol binding protein (RBP) and transthyretin (TTR) and circulates through the blood in this 1:1:1 complex. Retinol can be converted to retinoic acid, involved in growth and bone modulation. Ammended from Green and Green 1994.

authors concluded that dietary source was not a contributing factor to lower hepatic retinol concentrations. Boily et al. (1994) found retinyl palmitate in egg yolk of Great Blue Heron eggs to be negatively correlated with PCB 118 and PCB 105. However, retinoid precursors β–carotene and retinol were not correlated with contaminant concentrations, concluding that differences in vitamin A were not attributable to nutritional factors. Of all the carotenoids, β–carotene has the highest vitamin A activity (Green and Green 1994) and thus provides a good indication of dietary retinoids when conducting field studies.

The consequences of an impairment on the physiological performance remain to be assessed. It can be postulated however, that depressed levels of vitamin A could lead to reproductive impairment, depressed synthesis of immunoglobulins (contributing to lowered immune function), and reduced growth (Green and Green 1994).

Chapter 2

2.0. CONTAMINANT LEVELS IN TREE SWALLOWS (*TACHYCINETA BICOLOR*) NESTING ALONG THE ST. LAWRENCE RIVER, CANADA AND USA.

2.1. Introduction

Although persistent and bioaccumulative organochlorine contaminant levels in air, water and wildlife have declined globally since the 1970s (Bentzen et al. 1999), they are still an issue of concern in the Great Lakes and St. Lawrence River basins (Lean 2000). Environmental contaminants such as polychlorinated biphenyls (PCBs), polychlorinated dibenzodioxins (PCDDs), and polychlorinated dibenzofurans (PCDFs), and mercury occur in biota living in the St. Lawrence River and Akwasasne Mohawk Territory (Sloan and Jock 1990; Stone et al. 1991; Sokol et al. 1994). Potential contributors of PCBs to the St. Lawrence River Area of Concern (AOC), as identified by the International Joint Commission, include the Aluminum Company of America (ALCOA), Reynolds Metals and General Motors facilities discharging wastewater into the St. Lawrence River; a PCB landfill site in Akwasasne; and inactive hazardous waste sites associated with these three major industries, along the shores of the Grasse and Raquette River sub-basins where PCBs, metals, and organochlorine contaminants have been confirmed (Drier et al. 1997). In addition, volatilization and redeposition of PCB molecules from lakes and rivers have contributed to organochlorines in the St. Lawrence River (Bentzen et al. 1999).

Sediment levels as high as 956 μg/g dw have been reported for PCBs in the St. Lawrence River adjacent to the General Motors plant, and at 7956 μg/g dw at Reynolds

Metals outfalls close to the Grasse River (Sokol et al. 1994). Many passerine birds feed on insects that bioaccumulate organochlorine contaminants from sediment, such as mayflies (*Ephemeroptera*) and stoneflies (*Plecoptera*) (Tracey and Hansen 1996). Consequently, passerines such as tree swallows (*Tachycineta bicolor*) nesting along the shores of the St. Lawrence River AOC have been exposed to PCBs and PCDDs/PCDFs at high levels (Stone et al. 1991; Bishop et al. 1999). Similarly tree swallows have accumulated high PCBs (0.33 to 56 μg/g ww total PCB) from Denver, Colorado (DeWeese et al. 1985) and the Hudson River (McCarty and Secord 1997). In addition, mass at hatching in Hudson River tree swallows was lower in tree swallows measured from contaminated sites compared to reference sites River (McCarty and Secord 1997). Altered biochemical parameters have also been noted in tree swallows exposed to environmental contaminants. Bishop et al. (1999) found a positive correlation between carboxylated porphyrins and PCB 118 and a negative correlation between EROD and vitamin A in nestling tree swallows collected from the St. Lawrence River AOC and the Great Lakes. Nestlings contained up to 5.4 μg/g ww whole body total PCBs.

No studies have examined the levels of organochlorine contaminants in tree swallows nesting within the vicinity of all three industrial sites contributing to organochlorine pollution in the St. Lawrence River (Sokol et al. 1994). These include ALCOA, Reynolds Metals, and General Motors. Therefore the goal of this study was to determine levels of organochlorine contaminants in tree swallow nestlings along the shores of the St. Lawrence River AOC, within the vicinity of ALCOA, Reynold Metals, and General Motors.

The hypothesis for this study is that levels of lipophilic organochlorines (PCBs, dioxins, and furans) are significantly higher in tree swallow nestlings reared within the St. Lawrence AOC when compared with reference areas. Two additional chapters in this thesis investigate the effects of organochlorine contaminants on corticosterone and vitamin A (see Chapters 3 and 4).

2.2. Methods and Materials

2.2.1. *Study sites*

Tree swallow nesting boxes were erected and sampled along the St. Lawrence River in Canada and USA in an Area of Concern (AOC), and three sites outside the AOC in 1999. The highly contaminated AOC sites were Turtle Creek (44°59N, 74° 44'W), Grasse River North (44°59N, 74° 38'W), Grasse River South (44°59N, 74° 38'W), and the two less contaminated sites were Cornwall Island (44°59N, 74° 44'W), and Cornwall Sewage Treatment plant (45°52N, 74° 42'W). In 2000, an additional AOC site was included (Raquette River (44°59N, 74° 42'W)). The three non-AOCs were Hoasic Creek (44°57N, 57° 10'W), Upper Canada Bird Sanctuary (44°59N, 75° 01W), and Cooper Marsh (45°07N, 74° 31W). Upper Canada Bird Sanctuary was excluded in 2000. These sites were chosen to provide a gradient of contamination (Bishop et al. 1995; 1999, Stone et al. 1991).

2.2.2. *Tissue collection and preparation*

One 16 day old tree swallow was collected from each nest and euthanized by carbon dioxide asphyxiation, placed in a hexane-rinsed jar, and frozen at -20°C until chemical analysis.

Sample preparation for contaminant analysis involved thawing the nestlings and preparing the animal by first removing the feet, beak, and feathers. The chick was then cut into pieces and homogenized by blending in an Ultra-turrax homogenizer (Interscience 1KT25A) at high speed (approximately 2700 rpm) for 2 min. Portions of the homogenate (6.0 g) were transferred into OC clean glass jars, individually for total PCBs and organochlorine pesticides, and for dioxin and furan analyses, one pool of three chicks/site in 1999 and two pools of 3 chicks/site at all sites in 2000 excluding Grasse River N and S. Pooling of chicks for dioxin and furan analysis was necessary to meet the minimum requirement of 18.0 g for analysis. Pools consisted of 18.0 g for dioxin and furan analysis. A portion of each homogenate was used for mercury analysis and stored in polypropylene vials (McNeil 1997). All procedures were approved by the Canadian Wildlife Service Animal Care Committee.

2.2.3 Sediment and insect collections

Sediment was collected in 1999 with a ponar mini-dredge. Five samples of sediment ($30cm^2$) were collected within 2 metres of each other of the first 5-10 cm of surface sediment at all sites. Sampling sites were located close to tree swallow boxes. Each sample was mixed thoroughly to obtain a homogenous blend, and subsamples of 1 L were placed in separate acetone/hexane-rinsed teflon containers and stored at -20°C.

Adult aerial insects were collected from all sites in terrestrial light traps from Bio Quip (Gardena, CA) at dusk (>8 pm) over a 2 hour period between June 5th to June 10th. The traps were handled with latex gloves, and consisted of a five gallon plastic bucket with an aluminum funnel fitting directly into the bucket. A light source (12 V flourescent bulb) is supported by a plexiglass tripod structure and seated above the funnel. The invertebrates were kept at -20° C in OC clean jars and a subsample of 5.0 g was used for analysis. Between each sampling event, all light trap components (plexiglass vanes, funnel and aluminum foil) were rinsed with hexane to prevent cross contamination. Emergent insects collected in the evening were assumed to have similar contaminant burdens as insects collected during the day. Sampling during the day was not feasible to meet the minimum weight requirement (5.0 g). We chose for consistency of contaminant exposure in the organisms, and also to provide a method which collected greater than 10.0 g insects per site.

2.2.4 Contaminant Residue analysis

Organochlorine Pesticides and PCBs

Methods for measuring organochlorine pesticides and PCB congeners were the same as those described in Won et al. (2001). Briefly, for analysis the samples were thawed to room temperature and then homogenized (see previous page). A subsample of tissue homogenate (2.5 g) was dehydrated by grinding with an excess of anhydrous sodium sulfate (6:1) and extracted on a column with dichloromethane (DCM): hexane (1:1 v/v). The extract was evaporated to 2.5 ml volume and extracted on 8 g of 1.2% water deactivated Florisil column. This was eluted into three fractions (< 1ml each) and analyzed separately on a fused silica capillary column (60 m x 0.25 mm; 0.1 µm DB-5 film thickness). Each cleaned up sample was injected twice into a Gas Chromatograph. The first injection was designed to determine organochlorine (OC) pesticides (see appendix Table 1) by using twenty one OC standards; the second injection is to determine PCBs by using Aroclors 1254/1260, 1:1: quantitation standard mixture. The limit of detection for PCB congeners was 0.005 μg/g ww, and 0.0025 μg/g ww for organochlorine pesticides. Percent recoveries were included with the C^{13} labelled internal standards for Organochlorine contaminants/PCBs, and ranged from 70.09 to 103.42%. Lipids and biogenic materials were removed by Gel Permeation Chromatography, and further cleaned by Florisil column chromatography. The value of "total PCBs" reported here is the sum of the concentration of the following congeners measured individually in each sample: PCB 17, PCB 64, PCB 110, PCB 158, PCB 157, PCB 118, PCB 74, PCB 151, PCB 138, PCB 172, PCB 16/32, PCB 70/76, PCB 149, PCB 178, PCB 180, PCB 31, PCB 95, PCB 118, PCB 187, PCB 170/190, PCB 28, PCB 66, PCB 146, PCB 183, PCB 201, PCB 33/20, PCB 56/60, PCB 153, PCB 128, PCB 206, PCB 22, PCB 92, PCB 105, PCB 174, PCB 196, PCB 52, PCB 101/90, PCB 179, PCB 177, 203, PCB 49, PCB 99, PCB 141, PCB 202, PCB 208, PCB 47/48, PCB 97, PCB 130, PCB 171, PCB 195, PCB 44, PCB 87, PCB 176, PCB 156, PCB 207, PCB 42, PCB 85, PCB 137, PCB 200, PCB 194 (International Union of Pure and Applied Chemistry IUPAC #) (Ballschmiter and Zell 1980). Ratio of aroclor 1254:1260 was calculated based on PCB 138. Every 14.6 pg of PCB 138 is equivalent to 200 pg of aroclor 1254:1260. The reason for expressing this ratio is to allow comparisons to data collected and analysed between 1969-1985 (Turtle et al. 1991). chlorinated pesticides and benzenes measured include 1,2,4,5-TClBz (tetrachlorobenzene), 1,2,3,4-TClBz, PnClBz, α-hexachlorocyclohexane (HCH), hexachlorobenzene (HCB), β–HCH, γ-HCB, OCS (octachlorostyrene), Heptachlor (HE), oxychlordane, t-chlordane, c-chlordane, t-nonachlor, p,p'-DDE (dichlorodiphenyldichloroethylene), dieldrin, p,p'-DDE, c-nonachlor, p,p'-dichloro diphenyl trichlorethane (DDT), photomirex, mirex, TCPM (tris-chlorophenol methanol).

PCDDs, PCDFs, non-ortho PCBs, and Hg

Concentrations of total polychlorinated dibenzodioxins (PCDDs) (sum of TetraCDD, PentaCDD, HexaCDD, HeptaCDD, OctaCDD), total Polychlorinated dibenzofurans (PCDFs) (sum of TetraCDF, PentaCDF, HexaCDF, HeptaCDF, OctaCDF) and non-ortho PCBs (noPCBs) (PCB 37, PCB 81, PCB 77, PCB 126, PCB 169, and PCB 189) were measured in tree swallow nestlings collected from all sites in 1999 using capillary gas chromatography (GC) and mass spectroscopy (MS) (Norstrom and Simon 1991). Subsamples of the homogenate from each chick were pooled for each site and analyzed as a single sample. Sample preparation involved grinding a 5 g subsample of the sample with anhydrous sodium sulphate and extracting it with DCM/hexane (1:1 v/v). The sample was spiked with a primary internal standard mixture ($^{13}C^{12}$)-labeled PCDD/ PCDFs at a level of 50-100 ng/kg on top of the column prior to extraction. Prior to adjustment to the final volume of 10 µl, a secondary internal standard was added ($^{37}Cl^{4}$)-2378-tetrachloro-dibenzodioxin (TCDD). Lipids and biogenic materials were removed by Gel Permeation Chromatography, and further cleaned by Florisil column chromatography. Samples were analyzed with a Hewlett-Packard 5987B GC/MS (high resolution GC, low resolution MS), using a 30 m DB-5, thin-film, capillary GC column. The minimum detectable concentration for the PCDF and PCDD congeners varied from 0.1 to 3.9 ng/kg ww. Minimum detection limits for non-ortho PCBs ranged from 0.3 to

2.7 ng/kg ww. Percent recovery for PCDD/PCDF and non-ortho PCBs ranged from 79 to 125%.

Total mercury (inorganic and organic) was analyzed by cold vapour atomic adsorption according to Scheuhammer and Bond (1991). Samples were analyzed by CVTASS (VGA-76; Varian) at 253.7 nm wavelength and minimum detection limit was 0.001 µg/g ww (0.013 µg/g dry wt in tissue for 0.15 g dry wt sample). The sensitivity was 0.029 µg/g in the digest and % recovery was over 92.5.

All chemical residue analysis for PCBs, dioxin, furan, and Hg was performed by an accredited lab at the National Wildlife Research Centre, Environment Canada, Hull, Quebec.

Contaminants in sediment and insects

A five gram subsample of insects collected at each study site in 1999 was extracted with dichloromethane using a poltyron homogenizer. The concentrated extract was applied to a gel permeation column for removal of lipids. The extract was concentrated, cleaned, and fractionated on a 3% (w/w) deactivated silica gel column. It was then reconcentrated to a final volume of 10 ml prior to analysis. Packed column gas liquid chromatography with electron capture detector was used for PCBs. Prior to analysis, the sample was measured in a cellulose thimble and dried at 105°C to determine dry weight. The sample was also 'spiked' with a surrogate mixture and Soxhlet extracted with an acetone/hexane mix. Percent recovery ranged from 83-99%. The extract is back-extracted and exchanged to isooctane. 209 congeners of PCBs were analysed for total PCBs (see Appendix Table 2). Detection limits in sediment and insect were .023 µg/g (for both). All samples were analysed at the National Laboratory for Environmental Testing, Environment Canada, Canadian Centre for Inland Waters, Burlington, Ontario.

Toxic Equivalency Concentrations

The TEQ (toxic equivalency) approach has been used in risk assessment of PCDD/PCDF and PCBs, and is based on structure and affinity of the congener for the cytosolic Ah receptor. Congeners have been assigned a toxic equivalency factor (TEF), which is the fractional toxicity of the congener relative to the standard toxin, 2,3,7,8-TCDD (Ahlborg et al. 1992). TEF values can be derived by exposing cell cultures (like the H4IIE rat hepatoma culture (Safe 1994), or chicken embryo hepatocytes (Kennedy et al. 1996)) to a sample extract and measure the induction of enzymes known to respond to chlorinated hydrocarbons. Toxic Equivalency concentrations (TEQs) were calculated for non-ortho PCBs, PCDDs and PCDFs using congener specific toxic equivalency factors (TEFs) from laboratory rats (*Rattus rattus*) (Safe 1994) and chickens (*Gallus domesticus*) (Kennedy et al. 1996a; b).

2.2.5 Statistical analysis

All chemical data were tested for homogeneity of variance and normal distribution. Where these criteria were not met, non-parametric statistical tests were used. Kruskall-Wallis tests were used to determine differences among sites for total PCBs and organochlorine pesticides (Sokal and Rohlf 1981). The Tukey (for unequal sample size) post hoc test was used to determine differences among sites. Pooling of samples for PCDD, PCDF, and mercury precluded statistical testing of differences among sites in 1999 and 2000. Since a two-way ANOVA revealed differences in contaminant residues between years within sites, 1999 and 2000 were treated independently. Sediment (total organic carbon normalized), insect (lipid normalized) and tree swallow (lipid normalized) concentrations of total PCBs were used to calculate bioaccumulation factors (BAFs). Spearman rank correlations were performed between TOC and insect BAFs. Bioaccumulation factors were calculated for transfer of PCBs from sediment to biota. A significance value of $p=0.05$ was considered statistically significant in all analyses. All statistical analysis was conducted with Statistica version 5.7 software (Statsoft 1997).

2.3. Results

2.3.1 Contaminant levels - 1999 Tree swallow residues

Total PCBs were significantly different among sites ($p<0.001$, $H=40.15$), and were highest at Grasse River S (31.75 µg/g ww), followed by Turtle Creek (29.88 µg/g ww) and were moderately high at Grasse River N (10.41 µg/g ww) (Figure 2.1a; Appendix Table 3). p,p'-DDE concentrations were not significantly different among sites ($p=0.18$, $H=16.87$) (Figure 2.2a). Levels of p,p-DDE ranged from 0.01 µg/g ww (Hoasic Creek) to 0.09 µg/g ww (Cornwall Island). Concentrations of all other organochlorine pesticides ranged from 0.013 µg/g ww (Hoasic Creek) to 0.132 µg/g (Cornwall Island) ww among sites and were significantly different among sites ($p=0.0004$, $H=26.83$). Mean Aroclor 1254:1260 ratios and Aroclor 1260 were significantly different among sites ($p<0.001$, $H=38.60$; $p<0.001$, $H=36.45$) (Table 2.1).

All PCDD/PCDF, non-ortho PCBs and total mercury were analysed in one pool of three chicks/site. The highest concentration of non-ortho PCBs were found at Turtle Creek (159, 640 ng/kg ww) and Grasse River S (117, 137 ng/kg ww) (Figure 2.3a). The highest total PCDD concentration was in Grasse River N birds (79.51 ng/kg ww) (Figure

2.4a). The highest total PCDF was found at Turtle Creek (94.11 ng/kg ww) and Grasse River S (62.96 ng/kg ww) (Figure 2.5a; Appendix Table 4). Total mercury ranged from 0.05 (Cornwall Island) to 0.16 (UCBS) μg/g dw (dry weight) (Appendix Table 3).

2.3.2 PCB levels - Sediment and Insects - 1999

The highest level of PCBs in sediments was found at Turtle Creek (617.86 μg/g ww). Levels at Grasse River S were second highest (65.77 μg/g ww), followed by Grasse River N (34.60 μg/g ww). All other sites ranged from 0.32 ng/g (Cooper M) to 5.87 μg/g (Cornwall Island) (Table 2.2). Sediment types ranged from predominately gravel to organic matter (Table 2.2).

Insects from Turtle Creek also had the highest concentration of total PCBs (89.80 μg/g lw (lipid weight)), followed by Grasse River S (10.20 μg/g lw) and Grasse River N (7.70 μg/g lw) (Table 2.2). All other sites ranged from 1.30 μg/g lw (Upper Canada Bird Sanctuary) to 7.53 μg/g lw (Cornwall Island). Bioaccumulation factors (BAFs) for insects ranged from 0.14 at Turtle Creek to 19.19 at Cooper Marsh. In tree swallow chicks, the BAF ranged from 0.48 at Turtle Creek to 480 at Grasse River N (Table 2.2). No correlations were found between TOC and insect BAF. Insects at all sites were dominated by the order Stoneflies (*Plecoptera*) and Caddisflies (*Trichoptera*).

Toxic equivalent factors - 1999

TEQ values for total PCBs based on Kennedy et al. (1996) were highest at Turtle Creek (0.005 ng/kg ww) and Grasse River N (0.005 ng/kg ww). Non-ortho PCBs were highest at Turtle Creek with PCB77 predominating (2, 838 ng/kg ww). The next highest

TEQ values were for PCB 126 (1, 094 ng/kg ww). TEQ values for dioxin and furan residues were highest for 2,3,7,8 TCDF (Turtle Creek; 39.49 ng/kg ww) (Table 2.3).

For rat hepatocyte TEQ based on Safe (1994) the highest total PCB was found at Turtle Creek (0.0005 ng/kg ww) and Grasse River N (0.0005 ng/kg ww), and non-ortho PCB values were highest for PCB 126 at Turtle Creek (1, 094 ng/kg ww). For dioxin and furan, 2,3,7,8 TCDF was highest at Turtle Creek (3.23), and 2,3,7,8 TCDD was highest at Upper Canada Bird Sanctuary (1.96) (Table 2.4).

2.3.3 Contaminant levels - 2000 Tree swallow residues

Total PCB residues measured in whole body tree swallow nestlings showed significant differences among sites ($p<0.001$, $H=61.54$). Birds collected from Grasse River S contained the highest levels (69.13 μg/g ww). Similar residue concentrations were found at Grasse River N (58.64 μg/g ww), followed by Turtle Creek (51.59 ng/kg) (Figure 2.1b; Appendix Table 5). p,p'-DDE were not significantly different among sites (p0.078, $H=42.90$). Concentrations ranged from 0.01 μg/g ww (Hoasic Creek) to 0.03 μg/g ww (Grasse River N) (Figure 2.2b). The range of all total organochlorine pesticides (including p,p`-DDE) ranged from 0.005 μg/g ww to 0.061 μg/g ww, and were significantly different among sites ($p<0.001$, $H=47.83$) (Appendix Table 5). Ratio of Aroclor 1254:1260 and Aroclor 1260 were significantly different among sites ($p<0.001$, $H=62.22$; $p<0.001$, $H=61.06$).

All PCDD/PCDF, non-ortho PCBs and total mercury were analysed in two pools of three chicks/site (except Grasse River N and Grasse River S; n=1). non-ortho PCBs were highest at Turtle Creek (404 ng/g) followed by Grasse River N (150 ng/g) (Figure 2.3b).

Mean PCDD levels were highest at Grasse River N (32.40 ng/kg ww), Grasse River S (27.01 ng/kg ww) and the Raquette River (24.74 ng/kg ww) (Figure 2.4b). Turtle Creek chicks contained the highest levels of total PCDF (128.52 ng/kg ww), followed by Grasse River N (120.50 ng/kg ww) and Grasse River S (106.41 ng/kg) (Figure 2.5b). Mercury levels ranged from 0.04 µg/g dw (Cornwall Sewage Treatment Plant and Turtle Creek) to 0.20 µg/g dw (Hoasic Creek) (Appendix Table 5).

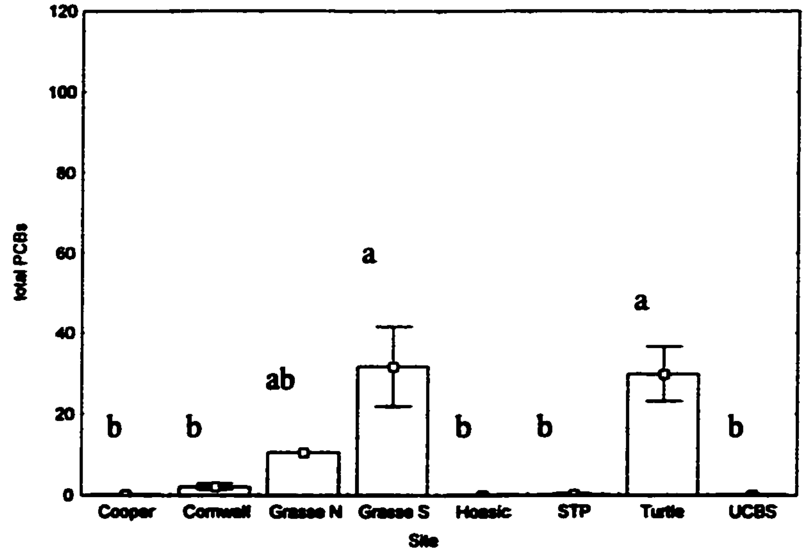
Toxic equivalent factors - 2000

TEQ (Kennedy et al. 1996) for total PCBs were highest at Turtle Creek (0.005 ng/kg ww) and Grasse River N (0.005). TEQ for non-ortho PCBs were highest for PCB 77 at Turtle Creek (5, 100 ng/kg). Residues of 2,3,7,8-TCDF accounted for the highest percentage of congener found for TCDF and PCDD (50.55 ng/kg ww for Turtle Creek) (Table 2.6). TEQ values based on rat hepatocyte induction for total PCBs were highest for Turtle Creek (0.001 ng/kg ww). PCB 126 was the highest non-ortho PCB at Turtle Creek (1245 ng/kg ww). TEQ values for dioxin and furans revealed that 2,3,7,8 TCDF (4.16 ng/kg at Turtle Creek) accounted for the highest percentage among most sites (Table 2.7).

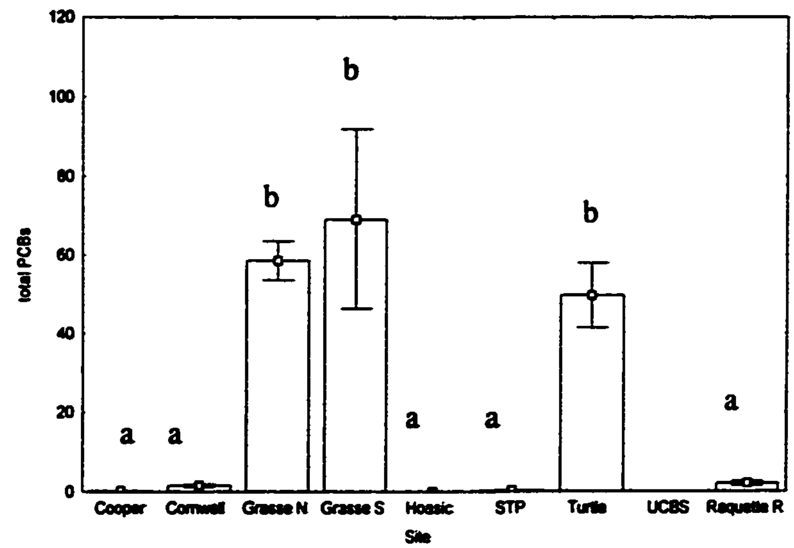
2.3.4. Between year differences for contaminant concentrations in tree swallows

Residues of total PCBs in birds from 2000 and 1999 contained a similar pattern of contamination with Grasse River S and Turtle Creek as the most contaminated sites, followed by Grasse River N in both years. However, the levels in 2000 were significantly higher than in 1999. Grasse River S was 31.75 µg/g ww in 1999, and then elevated to 69.13 µg/g ww in 2000. Turtle Creek and Grasse River N were 29.88 µg/g ww and 10.41

μg/g ww in 1999, respectively, and rose as high as 51.59 μg/g and 58.64 μg/g in 2000. Similarly levels of total PCDF were higher in 2000 for Turtle Creek (128.52 ng/kg) and Grasse River S (106.41 ng/kg) compared to 1999 (94.11 ng/kg and 62.96 ng/kg). In contrast, mean levels of total PCDD residues were higher in 1999 at Grasse River N (79.51 ng/kg), compared to levels in 2000 at Grasse River N (32.40 ng/kg).

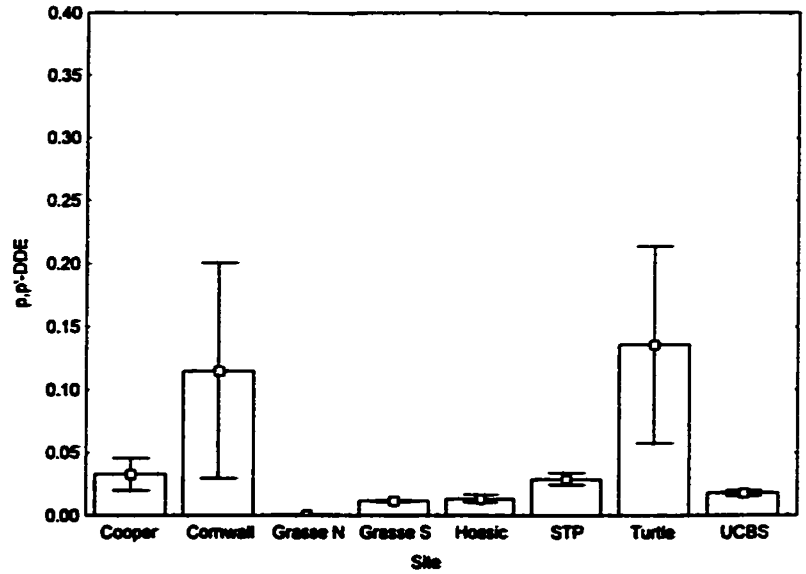


[A]



[B]

Figure 2.1. Mean total PCBs (µg/g ww) ± SE in 16 day old tree swallows collected from the St. Lawrence River in [A] 1999 and [B] 2000. Significant differences denoted by a,b (Tukey post hoc test, $p<0.05$) (1999: $p<0.001$, $H=40.10$; 2000; $p<0.001$, $H=61.50$). 1999; UCBS (7), Hoasic Creek (3), Grasse River N (2), Grasse River S (3), Turtle (7), Cooper (8), Cornwall STP (8), Cornwall Island (10). 2000 Sample sizes; Raquette River (10), Hoasic Creek (10), Grasse River N (4), Grasse River S (3), Turtle (10), Cooper (10), Cornwall STP (10), Cornwall Island (7).



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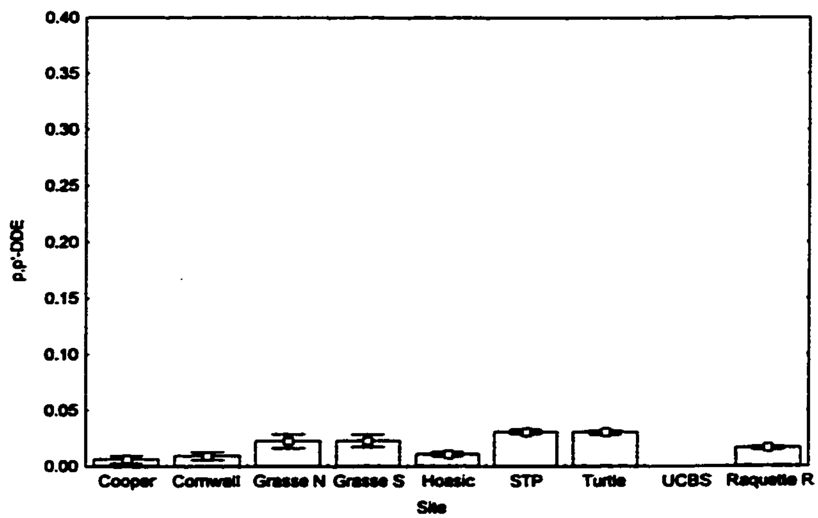
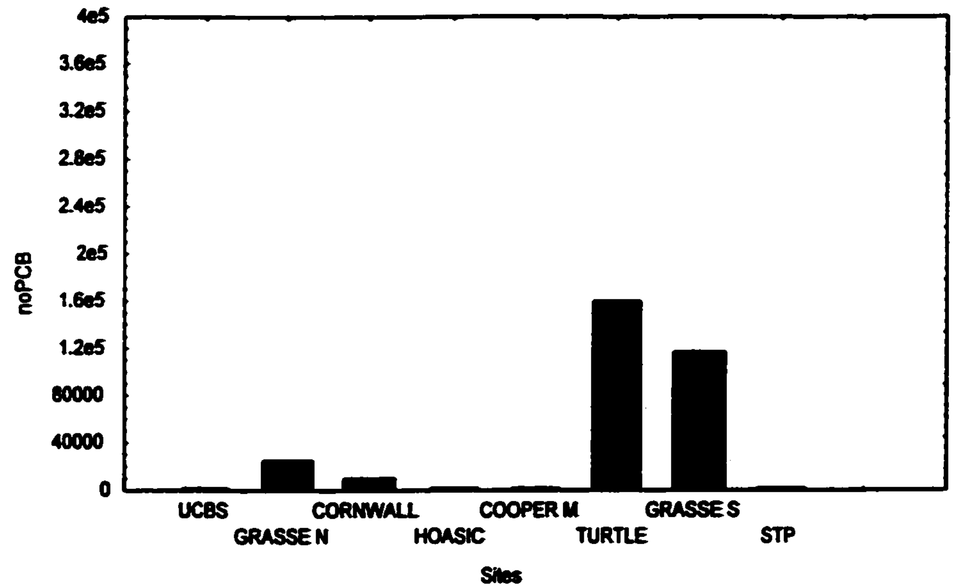


[B]

Figure 2.2. Mean p,p'-DDE (µg/g ww) ± SE in 16 d old tree swallows collected from the St. Lawrence River in [A] 1999 and [B] 2000. 1999; UCBS (7), Hoasic Creek (3), Grasse River N (2), Grasse River S (3), Turtle (7), Cooper (8), Cornwall STP (8), Cornwall Island (10). 2000; Raquette River (10), Hoasic Creek (10), Grasse River N (4), Grasse River S (3), Turtle (10), Cooper (10), Cornwall STP (10), Cornwall Island (7).



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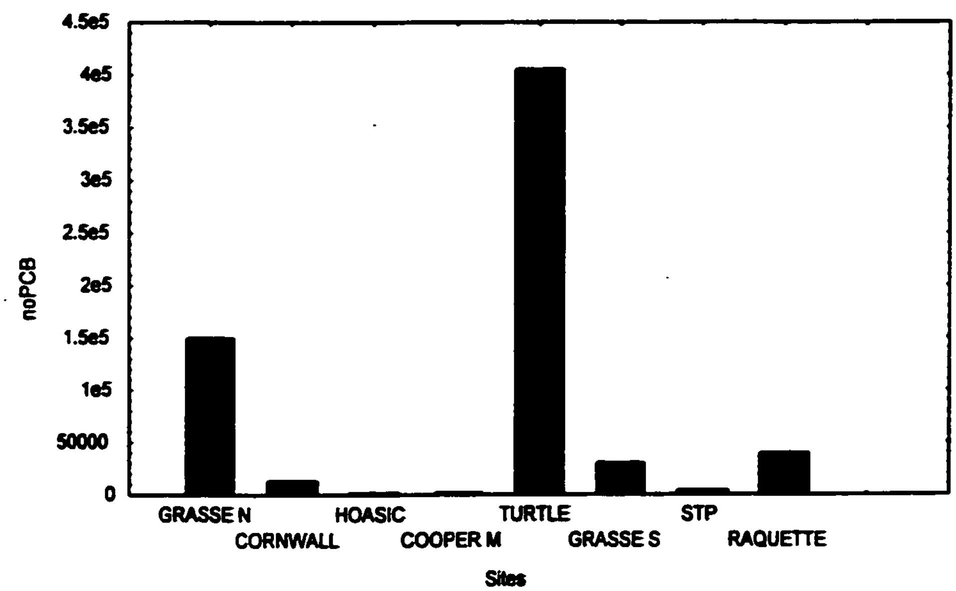
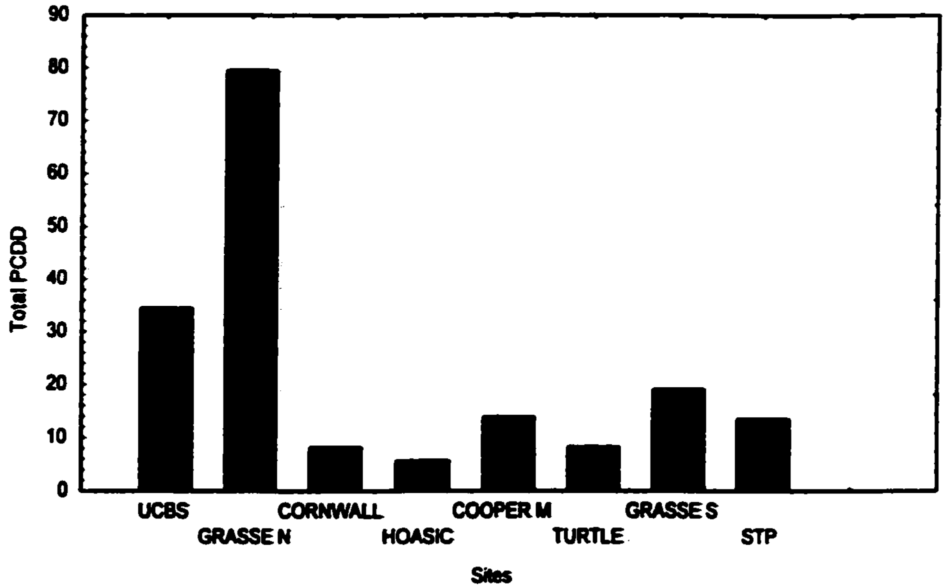


[B]

Figure 2.3. Pooled concentrations of non-ortho PCBs (sum of 77, 169, 126 and 37)(ng/kg ww) in 16 day old tree swallow nestlings, [A] 1999 (based on 1 pool of 3 chicks/site) and [B] 2000 (based on 2 pools of 3 chicks/site, except Grasse River N and S, n=1 pool).



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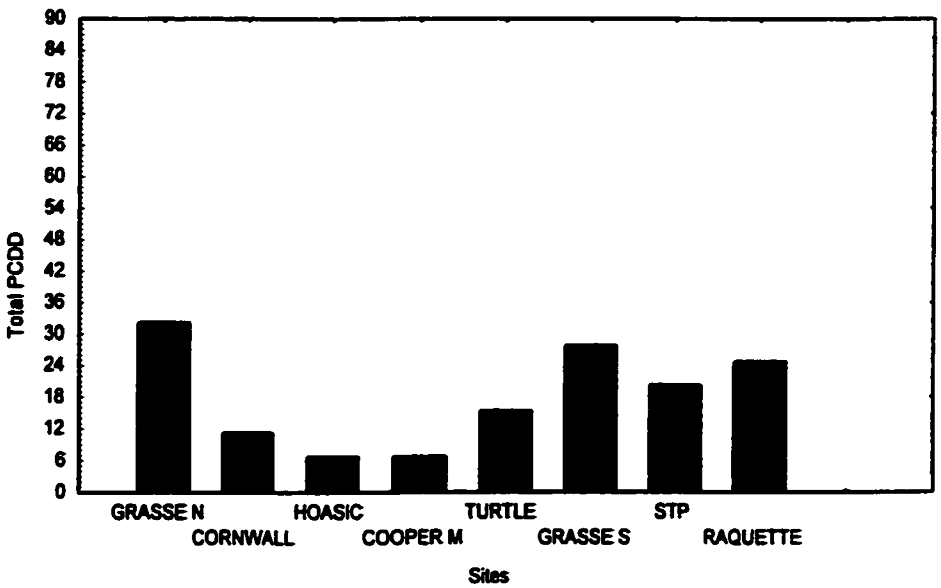
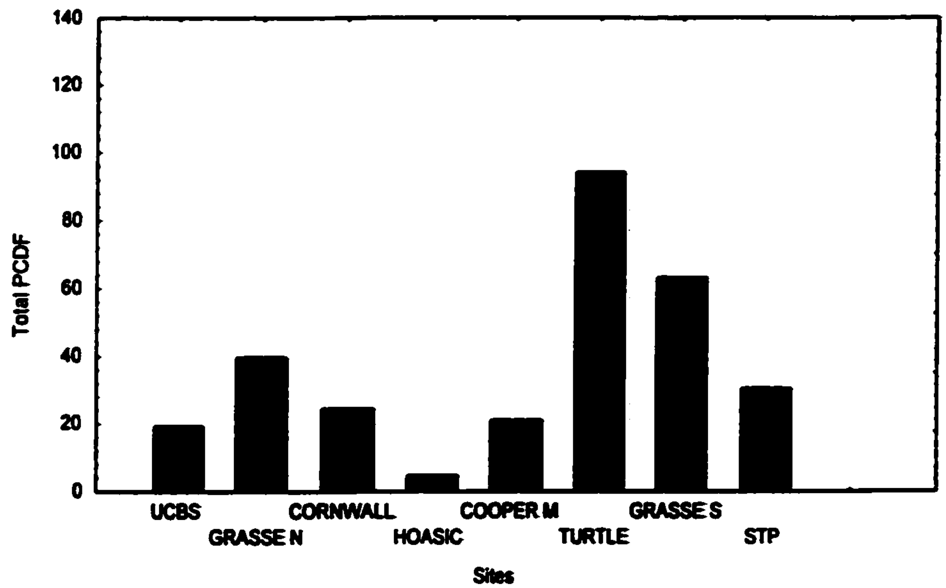


[B]

Figure 2.4. Pooled concentrations of total polychlorinated dibenzodioxin (PCDD*) (ng/kg ww) in 16 day old tree swallow nestlings in [A] 1999 (based on 1 pool of 3 chicks/site) and [B] 2000 (based on 2 pools of 3 chicks/site except Grasse River N and S n=1). Total PCDD includes sum of TetraCDD, PentaCDD, HeptaCDD, HexaCDD, OctaCDD based on 1 pool of 3 chicks/site for 1999 and 2 pools of 3 chicks/site for 2000.



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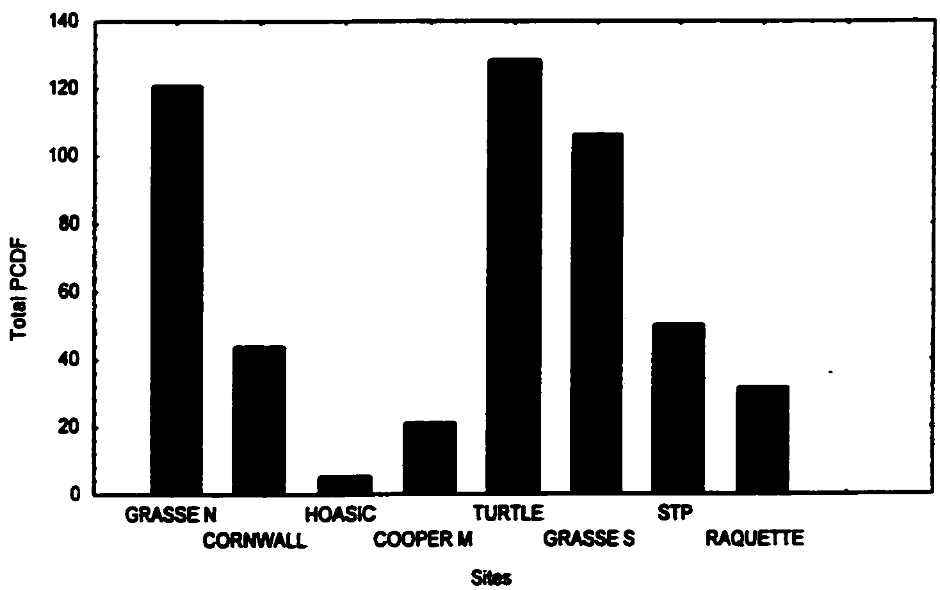


[B]

Figure 2.5. Pooled concentrations of total Polychlorinated dibenzofuran (PCDF*) (ng/kg ww) in 16 day old tree swallow nestlings in [A] 1999 (based on 1 pool of 3 chicks/site) and [B] 2000 (based on 2 pools of 3 chicks/site except Grasse River N and S n=1). Total PCDF includes sum of TetraCDF, PentaCDF, HeptaCDF, HexaCDF, OctaCDF based on 1 pool of 3 chicks/site for 1999 and 2 pools of 3 chicks/site for 2000.

Table 2.1. Aroclor 1254:1260 (µg/g ww) (mean ± standard deviation) and Aroclor 1260 (µg/g ww) (mean ± standard deviation) for 16 day old tree swallows from the St. Lawrence River.

1999

Site (*)	Aroclor 1254:1260[a] Mean	SD	Aroclor 1260[b] Mean	SD
UC Bird Sanctuary (c)	0.09 a	0.02	0.04 a	0.01
Grasse River N (c)	4.80 ab	0.00	3.00 cd	0.00
Cornwall Island (c)	0.91 b	0.56	0.44 abc	0.27
Hoasic Creek (r)	0.05 b	0.02	0.03 a	0.01
Cooper Marsh (r)	0.18 b	0.02	0.09 a	0.02
Turtle Creek (c)	7.08 a	4.90	2.18 d	1.44
Grasse River S (c)	7.76 a	1.93	3.58 d	0.76
Sewage Treatment P(c)	0.44 b	0.32	0.27 ab	0.31

* c - AOC site
* r - reference site
Significance set at $p<0.05$.

Table 2.2. Levels of total PCBs in sediment[c] (ng/g ww), insect (ng/g lw), and 16 d old tree swallows (ng/g lw), and bioaccumulation factor (BAF) from the St. Lawrence River basin.

1999

Site (*)	Sample	%TOC	sediment	total PCBs[a]	BAF[b]
Sewage Treatment P (c)	Sediment	0.7	gravel	2750.0	
Sewage Treatment P	Insect			3033.0	1.10
Sewage Treatment P	Tree swallow			5211.0	1.90
UC Bird Sanctuary (c)	Sediment	4.1	organic	850.0	
UC Bird Sanctuary	Insect			1300.0	1.53
UC Bird Sanctuary	Tree swallow			3005.0	15.30
Turtle Creek (c)	Sediment	1.4	organic	617857.0	
Turtle Creek	Insect		+	89800.0	0.14
Turtle Creek	Tree swallow		gravel	300000.0	0.48
Hoasic Creek (r)	Sediment	8.9	organic	666.3	
Hoasic Creek	Insect			1633.9	2.45
Hoasic Creek	Tree swallow			400.0	0.60
Cornwall Island (c)	Sediment	1.1	gravel	5872.7	
Cornwall Island	Insect		+	7533.0	1.28
Cornwall Island	Tree swallow		rock	16700.0	28.00
Grasse River S (c)	Sediment	1.3	gravel	65769.2	
Grasse River S	Insect			10200.0	0.15
Grasse River S	Tree swallow			246200.0	3.70
Grasse River N (c)	Sediment	2.5	gravel	34600.0	
Grasse River N	Insect			7700.0	0.22
Grasse River N	Tree swallow			111100.0	480.00
Cooper Marsh (r)	Sediment	7.1	organic	323.0	
Cooper Marsh	Insect			6200.0	19.19
Cooper Marsh	Tree swallow			1800.0	5.50

* c - AOC site
* r - reference site
ND - not detectable
total PCBs[a] - sum of PCB congeners (see Chapter 1 methods)
BAF[b] -Bioaccumulation factor for PCB accumulation (ng/g lipid normalized) from sediment (DOC normalized)
TOC total organic carbon
sediment[c] - dissolved organic carbon normalized
all sediment and insect samples based on 1 pool/site
Dominant family of insect were Stoneflies and Caddisflies at all sites sampled.

Table 2.3. Mean TEQ values based on Kennedy et al. (1996) chicken embryo hepatocyte induction for 16 day old tree swallows from the St. Lawrence River.

	1999								
Site (*)	PCB 77[b]	PCB 169[b]	PCB 126[b]	PCB 37[b]	Dioxin 2378[b]	Dioxin 123478[b]	Furan 2378[b]	Total PCB[a]	TEQ sum
UC Bird Sanctuary (c)	14.1	0.120	15.9	0.039	1.96	6.24	5.28	0.0000	43.63
Grasse River N (c)	570.4	0.560	248.7	1.791	.76	2.78	11.66	0.0046	836.65
Cornwall Island (c)	246.5	0.140	71.4	0.523	.94	1.64	11.77	0.0005	332.91
Hoasic Creek (r)	6.2	0.040	5.7	0.024	.37	0.63	1.24	0.0000	14.20
Cooper Marsh (r)	20.4	0.080	19.8	0.045	1.08	2.54	7.92	0.0001	51.86
Turtle Creek (c)	2838.8	0.760	1094.1	22.473	1.01	1.42	39.49	0.0049	3998.05
Grasse River S (c)	2115.2	0.740	692.1	16.610	.57	4.54	21.56	0.0008	2851.32
Sewage Treatment Plant (c)	36.4	0.160	30.0	0.079	1.61	2.38	15.62	0.0001	86.24

[a] Total PCBs (polychlorinated biphenyls) based on sum of congeners 105, 118, 156, 157, 47 and 153.
* c - AOC site
* r - reference site
samples based on 1 pool of 3 chicks/site
NoPCB - sum of PCB 77, 169, 126 and 37 (ng/kg)
PCDD - sum of individual PCDD congeners (tetraCDD, penta CDD, hexaCDD, heptaCDD, octaCDD)
2378D - 2,3,7,8-tetrachlorinated dibenzodioxin
12378D - 1,2,3,7,8-pentachlorinated dibenzodioxin
PCDF - sum of ndividual PCDF congeners (tetraCDF, penta CDF, hexaCDF, heptaCDF, octaCDF)
2378F - 2,3,7,8 - tetrachlorinated dibenzofuran
[b] Mean levels in ng/kg ww.
[c] Mean levels in µg/g ww.

Table 2.4 Mean TEQ values based on Safe (1994) rat hepatocyte induction for 16 day old tree swallows from the St. Lawrence River.

1999

Site (*)	PCB 77[b]	PCB 169[b]	PCB 126[b]	Dioxin 2378[b]	Dioxin 123478[b]	Furan 2378[b]	Total PCB[ac]	TEQ sum
UC Bird Sanctuary (c)	0.42	0.018	15.90	1.96	0.057	0.43	0.00000	18.78
Grasse River N (c)	17.11	0.084	248.70	0.76	0.025	0.95	0.00050	267.62
Cornwall Island (c)	7.40	0.021	71.40	0.94	0.015	0.96	0.00005	80.73
Hoasic Creek (r)	0.19	0.006	5.70	0.37	0.006	0.10	0.00000	6.37
Cooper Marsh (r)	0.61	0.012	19.80	1.08	0.023	0.65	0.00001	22.17
Turtle Creek (c)	85.17	0.114	1094.10	1.01	0.013	3.23	0.00053	1183.63
Grasse River S (c)	63.45	0.111	692.10	0.57	0.016	1.76	0.00007	758.00
Sewage Treatment Plant (c)	1.09	0.024	30.00	1.61	0.022	1.28	0.00001	34.02

[a] Total PCBs (polychlorinated biphenyls) based on sum of congeners 105, 118, 156, 157, 47 and 153.
[b] Mean levels in ng/kg ww.
[c] Mean levels in µg/g ww.
* c - AOC site
* r - reference site

Table 2.5. Aroclor 1254:1260 (µg/g ww) (mean ± standard deviation) and Aroclor 1260 (µg/g ww) (mean ± standard deviation) for 16 day old tree swallows from the St. Lawrence River.

2000

Site (*)	Aroclor 1254:1260 Mean	Aroclor 1254:1260 SD.	Aroclor 1260 Mean	Aroclor 1260 SD.
Grasse River N (c)	16.73	3.42	10.28	2.17
Cornwall Island (c)	0.79	0.30	0.34	0.12
Hoasic Creek (r)	0.02	0.01	0.01	0.01
Cooper Marsh (r)	0.22	0.03	0.10	0.01
Turtle Creek (c)	10.70	5.10	3.62	1.49
Grasse River S (c)	17.20	8.81	10.49	5.20
Sewage Treatment P (c)	0.34	0.04	0.13	0.03
Raquette River (c)	1.04	0.40	0.34	0.17

* c - contaminated site
* r - reference site
Aroclor 1254:1260[a] - Aroclor (1:1) calculation based on PCB138 result. Every 14.6 pg of PCB138 is equivalent to 200 pg of Aroclor 1254:1260, total.
Aroclor 1260[b] - Aroclor (1:1) calculation based on PCB180 result. Every 10.96 pg of PCB180 is equivalent to 100 pg of Aroclor 1260.

Table 2.6. Mean TEQ values based on Kennedy et al. (1996) chicken embryo hepatocyte induction for 16 day old tree swallows from the St. Lawrence River.

2000

Site (*)	PCB 77[b]	PCB 169[b]	PCB 126[b]	PCB 37[b]	Dioxin 2378[b]	Dioxin 123478[b]	Furan 2378[b]	Total PCB[ac]	TEQ sum
Grasse River N (c)	2280.0	1.98	630.0	18.80	1.28	2.81	45.10	0.0046	2979.90
Cornwall Island (c)	247.5	0.25	78.0	3.16	1.42	3.67	18.43	0.0005	352.43
Hoasic Creek (r)	4.2	0.06	4.7	0.05	0.26	0.72	1.19	0.0000	11.18
Cooper Marsh (r)	36.0	0.12	21.6	0.24	0.89	1.04	8.96	0.0001	68.85
Turtle Creek (c)	5100.0	1.10	1245.0	88.0	1.30	1.76	50.55	0.0049	6487.70
Grasse River S (c)	NA	NA	NA	NA	1.21	2.23	3.66	0.0008	7.10
Sewage Treatment P (c)	57.0	0.23	40.5	0.70	2.16	2.68	21.62	0.0001	124.89
Raquette River (c)	567.0	0.21	128.0	7.72	0.79	1.42	10.31	0.0000	715.45

[a] Total PCBs (polychlorinated biphenyls) based on sum of congeners 105, 118, 156, 157, 47 and 153.
* c - contaminated site
* r - reference site
samples based on 2 pools of 3 chicks/site (except Grasse River N and S, n=1).
NoPCB - sum of PCB 77, 169, 126 and 37 (ng/kg)
PCDD - sum of individual PCDD congeners (tetraCDD, penta CDD, hexaCDD, heptaCDD, octaCDD)
2378D - 2,3,7,8-tetrachlorinated dibenzodioxin
12378D - 1,2,3,7,8-pentachlorinated dibenzodioxin
PCDF - sum of individual PCDF congeners (tetraCDF, penta CDF, hexaCDF, heptaCDF, octaCDF)
2378F - 2,3,7,8 - tetrachlorinated dibenzofuran
[b] Mean levels in ng/kg ww.
[c] Mean levels in µg/g ww.
NA - not available

Table 2.7. Mean TEQ values based on Safe (1994) rat hepatocyte induction for 16 day old tree swallows from the St. Lawrence River.

2000

Site (*)	PCB 77[b]	PCB 169[b]	PCB 126[b]	Dioxin 2378[b]	Dioxin 123478[b]	Furan 2378[b]	Total PCB[ac]	TEQ
Grasse River N (c)	6.84	0.297	630.0	1.28	0.026	3.69	0.00017	642.13
Cornwall Island (c)	0.74	0.037	78.0	1.42	0.014	1.50	0.00003	81.71
Hoasic Creek (r)	0.01	0.009	4.7	0.26	0.007	0.09	0.00000	5.08
Cooper Marsh (r)	0.11	0.017	21.6	0.89	0.009	0.73	0.00001	23.31
Turtle Creek (c)	15.30	0.165	1245.0	1.30	0.016	4.16	0.00101	1265.90
Grasse River S (c)	NA	NA	NA	1.21	0.020	0.30	0.00022	1.53
Sewage Treatment P(c)	0.17	0.035	40.5	2.16	0.024	1.76	0.00001	44.65
Raquette River (c)	1.70	0.032	128.0	0.79	0.013	0.84	0.00005	131.37

* c - contaminated site
* r - reference site
[a] Total PCBs (polychlorinated biphenyls) based on sum of congeners 105, 118, 156, 157, 47 and 153.
[b] Mean levels in ng/kg ww.
[c] Mean levels in µg/g ww.
NA - Not Available

2.4. Discussion

These results found significant spatial differences in organochlorine contaminant concentrations in 16 day old tree swallows nesting along the shores of the St. Lawrence River Area of Concern. The highest concentrations of total PCBs, non-ortho PCBs, and PCDF were found at Turtle Creek, Grasse River S and Grasse River N. Despite a 2 km distance between Grasse River N and S, dioxins were 4 times higher at Grasse River N compared to Grasse River S. The highest level of PCDD was also found in tree swallow nestlings from Grasse River N.

Tree swallow chicks from Turtle Creek, Grasse River N and Grasse River S had the highest level of PCBs among all the sites. Higher PCB body burden in nestlings from Turtle creek probably reflect past disposal practices at General Motors. Historically, General Motors used PCB-containing hydraulic fluids in their diecasting machines from 1959 to 1974. PCB-contaminated sludge was disposed in several areas on GM property and within an industrial landfill that is known to have leached into an adjacent bay connecting to the St. Lawrence River (Stone et al. 1991), adjacent to the tree swallow boxes. Nestlings collected from Grasse River S and Grasse River N probably indicate contamination from ALCOA and Reynolds metals. The Aluminum Company of America plant, a state superfund site, is located approximately 10 km west of General Motors, upstream of the Grasse River. At ALCOA, the leakage of PCB-containing hydraulic and transfer fluids has occured in the past. Reynolds Metals Company, a State Superfund site located on the St. Lawrence River just west of General Motors, historically released heat transfer fluids containing PCBs (NYSDEC 1990; Chiarenzelli et al. 2000).

Congener patterns for non-ortho PCBs showed PCB 77 and 37 accounted for the highest percent of non-ortho PCBs. PCB 77 (3,3',4,4' tetrachlorobiphenyl) and PCB 37 (3,4,4' trichorobiphenyl) are congeners which are laterally substituted that do not have two adjacent unsubstituted carbon atoms. Since these characteristics contribute to slower metabolism and higher accumulation in animals (Safe 1994), tree swallows are likely accumulating these congeners to a higher degree than other PCB congeners.

Tree swallow nestlings from Turtle Creek, Grasse River S and Grasse River N also contained higher levels of Aroclor 1260 compared to other sites along the St. Lawrence River AOC and reference sites. Aroclor 1260 was a commercial mixture of PCB (containing 60% chlorine by weight) that was widely used as heat exchange and hydraulic fluids, and dielectric fluids in transformers and capacitors. Higher chlorinated congeners in sediment samples from Reynolds Metals, General Motors and ALCOA, which matched the Aroclor 1260 profile, have also been found (Sokol et al. 1994). Since the three Superfund industries, ALCOA, Reynolds Metals, and General Motors historically utilized this PCB mixture (Sokol et al. 1994) levels of Aroclor 1260 in tree swallow nestlings adds further evidence to a local PCB source.

Total PCDDs were highest in tree swallow nestlings at Grasse River N. The highest levels of PCDFs were found in chicks collected from Turtle Creek and Grasse River S. According to reports from the New York State Department of Environmental Conservation (NYSDEC), there are no known or permitted discharges of dioxin/ furan in the St. Lawrence River basin. However, PCDD and PCDF have been detected in soil samples taken from the Reynolds Metals property (Stone et al. 1991). Historically, the presence of chlorobenzene in dielectrical fluids presented a risk for the production of

PCDD, and electrical fires or overheating could also have led to the conversion of PCB transformer fluid to PCDFs (Safe 1994). A Canadian national inventory of toxic substances reported in 1982 that there were at least 1.7×10^7 kg of PCBs in Canada, which could include 85 kg of PCDFs, and up to 50% as the highly toxic congener 2,3,7,8-TCDF (Mitchell et al. 1984). Therefore, the presence of industries which used PCBs in the past, may also have contributed to dioxin and furan contamination of the St. Lawrence River AOC, and consequently bioaccumulation into tree swallow nestlings.

Concentrations of environmental contaminants found in tree swallow chicks from the St. Lawrence River are higher than tree swallows nesting in the Great Lakes basin. Tree swallow chicks collected from the Saginaw River ranged from 0.17 to 1.03 μg/g total PCB ww (Nichols et al. 1995), which is comparable to nestlings from five other sites around Lakes Erie, Huron, and Ontario with levels ranging from 0.26-1.01 μg/g total PCB ww (Bishop et al. 1995). Nestlings reared in Green Bay accumulated 13.10 μg/g total PCBs (Jones et al. 1993). At other locations in the United States and Canada, PCBs ranged between 0.25 and 0.33 μg/g in tree swallow eggs in Colarado (DeWeese et al. 1985) and 0.18 μg/g in eggs in British Columbia between 1990-1991 (Elliott et al. 1994). Along the Hudson River in New York, mean levels of PCBs in tree swallow nestlings were as high as 62.2 μg/g ww (McCarty and Secord 1999) compared with tree swallows nestlings from Grasse River S (69.1 μg/g ww) and Turtle Creek (51.6 μg/g ww).

In contrast, organochlorine pesticides including p,p'-DDE concentrations in chicks were found in much lower concentrations than other sites in Canada. The highest concentration of organochlorine pesticides in the St. Lawrence River chicks was p, p'-

DDE. Mean concentrations of p,p`-DDE ranged from 0.01 to 0.09 µg/g ww among study sites. In the Great Lakes in 1991, levels ranged from 0.5 to 4.3 µg/g dw in eggs and 0.06 µg/g dw in tree swallow nestlings (Bishop et al. 1995). However, levels in tree swallows from the St. Lawrence River were higher than in 10 day old tree swallow nestlings from central Alberta (0.31 µg/g) (Shaw 1983). Tree swallows nesting in non-orchards had relatively higher levels (3.29 µg/g ww) in eggs compared to residues in orchards (9.44 µg/g) in British Columbia (Elliott et al. 1994).

In their larval stages aquatic insects are in contact with sediments, and here emerging insects can transport contaminants to aquatic and terrestrial food chains (Fairchild et al. 1992). Highest levels of total PCBs were found in sediment and insects from Turtle Creek and Grasse River which is similar to the pattern for tree swallow nestlings.

Numerous factors may account for contaminant differences between sediment, insects and tree swallows. Contaminant accumulation rates for insects and tree swallows may be a factor of bioavailability. The low BAF for insects seen at most sites may be in part due to differences of lipophilic compounds in the organic carbon on the sediment versus the lipid of insects. Biota sediment accumulation factors (BASFs) are also dependent on the chemicophysical properties of both the residue and sediments. In the St. Lawrence River numerous chemicals may be bound together in the sediment, potentially making some compounds unavailable to invertebrates (Chiarenzelli et al. 2000). Differences were found in sediment type among study sites which may contribute to differences observed in contaminant accumulation in insects and tree swallow nestlings.

Gravel substrate, typically found in the St. Lawrence River at Cornwall Island and Cornwall Sewage Treatment Plant may not bind contaminants as tightly as organic matter, the substrate typically found at Cooper Marsh, Hoasic Creek, Upper Canada Bird Sanctuary and Turtle Creek. Contaminants have a higher retention time in organic and clay type sediments typically associated with high carbon content (Cho et al. 2000). We found total carbon varied from 0.75% (Cornwall Sewage Treatment Plant) to 8.9% (Hoasic Creek) among sites. Other sites with low total organic carbon, Cornwall Island (1.1%) and Grasse River S (1.3%), also contained the highest proportion of gravel and rock sustrate. Study sites with higher total organic carbon such as Cooper Marsh (7.1%) and Upper Canada Bird Sanctuary (4.1%) contained substrates mainly composed of organic matter in wetlands with slow moving or stagnant water. However, there was no correlation between TOC and insect or tree swallow BAF based on pooled samples per site.

During the breeding season tree swallows tend to concentrate on small (<10 mm) adult insects of the order Diptera and suborder Homoptera (Quinney and Ankney 1985). Similarly different invertebrate communities will have different BAFs for total PCBs. For example, mayfly (*Hexagenia limbata*) had a BAF of 0.8, and midge (*Chironomid spp*) were 5.3 (Tracey and Hansen 1996) for PCBs. Differences between BAFs may be due to the substrate habitat which each organism spends its larval stage in, and also the ability of these insects to metabolize organochlorine contaminants will utimately affect their contaminant load. Therefore, composite samples of emergent invertebrates which were collected in this study combine the overall exposure of the organisms to an area in which actual concentrations of residues, such as type of sediment, total organic carbon content, and PCBs can be heterogeneous (Froese et al. 1998). This may explain the similarities seen in insects collected from Grasse River N and Cooper Marsh. Although Grasse River N tree swallow chicks and sediment contained high levels of PCB (111,000 μg/g lw and 34,600 ng/g ww, respectively) compared to Cornwall Island (16,700 μg/g lw and 5,872 ng/g ww, respectively), levels for insects were similar for both sites (7,700 for Grasse River N and 7,533 for Cornwall Island).

Large difference in contaminant residues between years was observed for tree swallows nesting along the St. Lawrence River basin and surrounding tributaries. Levels of PCBs, non-ortho PCBs, and furans were higher in 2000 than in 1999 at most sites in the AOC. Similar trends for PCDD and PCDF have been observed for herring gull eggs throughout the Great Lakes among individual lakes and congeners (Hebert et al 1994). These widespread irregularities may be the result of basin-wide fluctuations affecting ecological factors such as food availability and invertebrate community composition (Safe 1994). For example, Cho et al. (2000) found differences in dechlorinating populations of microorganisms between sampling sites at General Motors, ALCOA, and Reynolds Metal, capable and not capable of removing meta position chlorine atoms from biphenyl rings. Consequently, less bioaccumulative forms of PCBs, which are more readily available for air-water exchange, predominated the St. Lawrence River basin. Shifts in the dynamics of microorganism communities may therefore affect sediment levels of PCBs available for bioaccumulation. In 1999 the total rainfall for the St. Lawrence River basin was 70.9 cm for June versus 100 cm in 2000. Heavier precipitation may increase leaching of contaminants from landfill sites or increase sediment suspension changing BAFs in insects and tree swallow chicks.

Changes in contaminant dynamics may also be the result of increased or decreased sediment resuspension. Evidence of this was seen in caspian terns (*Sterna caspia*) after a massive storm event in 1986 led to reduced reproductive success in Saginaw Bay. The 35.1-44.7 cm of rain in 30 hr contributed to mobilized sediments from the Saginaw River to the bay attributing to peaks in TCDD and PentaCDF levels in herring gull eggs from the same island in 1987 (Ludwig et al. 1993). However as far as is known no major shift in sediments occured at the Turtle Creek and Grasse River N and S sites between 1999 and 2000. In 1999 the total rainfall for the St. Lawrence River basin was 70.9 cm for June versus 100 cm in 2000. Heavier precipitation may increase leaching of contaminants from landfill sites or increase sediment suspension changing BAFs in insects and tree swallow chicks.

Chlorinated hydrocarbons can cause a number of adverse effects, among the most ecologically relevant being embryo-mortality and birth defects, which appear to be mediated through the Ah-receptor (Safe 1994). The potency of individual congeners can thus be reported relative to 2,3,7,8-TCDD which is the most toxic congener. In this study, TEQ values for non-ortho PCB 77 (5000 ng/kg) based on Kennedy et al. (1996a; 1996b) accounted for the highest amount of environmental non-ortho PCB congeners among the sites, and was found at Turtle Creek. Chlorine atoms on PCB 77 (3,3',4,4' tetraCB) are situated at the meta and para position, contributing to their ability to strongly bind to the aryl hydrocarbon receptors and induce dioxin like effects (deVoogt et al. 1990) including reproductive impairment, estrogenic and antiestrogenic effects, and increased rate of deformities (Safe 1994). PCB 77 TEQs of 0.008 pg/g in chick embryos was sufficient to inhibit bursal lymphoid development in lab studies (Safe 1994). Herring gull (*Larus argentatus*) embryos collected from the Great Lakes with 2,3,7,8-TCDD TEQ values of 1200 ng/kg were linked to lower basal levels of corticosterone (Lorenzen et al. 1999).

In contrast to chicken hepatocyte EROD induction, TEFs using rat hepatocyte induction by Safe (1994) indicated PCB 126 as the primary contributor to non-ortho TEQs. Laboratory studies have found TEQ for PCB 126 in mice to be teratogenic at 0.07 pg/g, and cause thymic atrophy and body weight loss at 0.015 pg/g to 0.093 pg/g (Safe 1994). TEQ levels determined for tree swallow nestlings may be sufficient to cause biochemical alterations in certain avian species. However, it is difficult to draw definitive conclusions about the actual toxicity of the contaminant mixture to which St. Lawrence tree swallows are exposed due to limitations of the TEQ approach for ecological risk assessment.

In this study, there were differences among TEQ values when comparing chicken-embryo hepatocyte culture against H4IIE rat hepatoma cell bioassay. The TEQs calculated from chicken embryo hepatocyte culture (Kennedy et al. 1996) resulted in higher values compared to those based on rats (Safe 1994). This method of establishing TEQ values utilizes chickens as the study species, which tend to be highly susceptible to PCB and dioxin exposure, and more sensitive to EROD induction by TCDD compared to pheasants, turkeys, ducks, or herring gulls (Kennedy et al. 1996a). Interestingly, the values for the current study did not find extreme differences between the two methods for PCB 126, but did find that PCB 77 was the dominant TEQ value for chicken embryo hepatocyte induction compared to rat hepatocyte induction, indicating differences in potencies of the non ortho congeners. Investigations on the assessment of TEQs suggested that underestimation could be the result of cytotoxic effects from some of the extracts, while overestimation was probably the result of the presence of Ah-receptor-active congeners in the extracts that were not quantified in the instrumental determinations. Predictive values of TEQs for PCBs may be both species- and response-dependent because both additive and non-additive interactions have been observed with PCB mixtures (Safe 1994).

2.5. Summary and Conclusions

Levels of total PCBs, dioxins, and furans in tree swallow nestlings are some of the highest reported for avian species throughout Canada and the United States. High levels of total PCBs, non-ortho PCBs, and 2,3,7,8-TCDF were found at Grasse River and Turtle Creek, within the vicinity of ALCOA and GM properties. Temporal differences in chlorinated hydrocarbon accumulation existd between years within sites. Similarly, BAF varied among sites for insects/sediment and tree swallows/sediment. The reasons for this variaiton cannot be fully explained at this point but suggest variation in tree swallow prey items sampled among sites. Changes in leaching and outflow of contaminants could also account for interyear differences in contaminant concentrations in tree swallow nestlings. Given the high concentration of contaminants found in sediment and biota, it is conceivable that biochemical parameters may also be altered in animals residing within the vicinity of these industrial sites.

Chapter 3

3.0. CORTICOSTERONE LEVELS IN TREE SWALLOW *(TACHYCINETA BICOLOR)* NESTLINGS EXPOSED TO A GRADIENT OF ENVIRONMENTAL CONTAMINATION.

3.1. Introduction

Glucocorticoids, such as corticosterone, aid in the mediation of critical processes such as gluconeogensis, allowing an animal to meet increased energy demands during stressful situations (Colby and Longhurst 1996). However, responding to a stressor can interfere with other physiological processes such as digestion, growth, immune function, and reproduction. Higher circulating levels of corticosterone can lead to immunosuppression, reduced release of reproductive hormones, and decrease protein synthesis leading to loss of muscle tissue (Norris 1997). Similarly, lower circulating levels of corticosterone can result in an inability to respond to stress, reduce gluconeogenesis and stimulate the immune system (Colby and Longhurst 1996). Environmental pollutants can disrupt this endocrine pathway and provoke a similar adrenal response to natural stressors. Consequently, corticosterone has been identified as potentially useful as a bioindicator of general toxic stress (Hontela 1997).

The adrenal gland is highly susceptible to chemically induced morphological damage. The cortex of the adrenal gland is rich in fat, resulting in the deposition and accumulation of lipophilic xenobiotics (Ribelin 1984). In addition, the adrenal gland has the capacity to metabolize toxicants through bioactivation of adrenal monooxygenases (P450 family), in some cases producing toxic metabolites (for example hydroxylated PCBs). The majority of studies have focused on teleost fish (Hontela et al. 1992) and found general suppression of basal corticosterone in response to environmental contaminants (PCBs, PAHs, metals). In amphibians the corticosterone response to environmental contaminants has been studied in only two species, mudpuppy (*Necturus maculosus*; Gendron et al 1997) and southern toads (*Bufo terrestris*; Hopkins et al 1999). In mudpuppies, depression of stress corticosterone response correlated with PCB exposure in the St. Lawrence River basin. In southern toads, higher circulating levels of basal corticosterone and testosterone were found at sites polluted with coal combustion waste. Few studies have described corticosterone levels in wild birds exposed to pollution. In herring gull (*Larus argentatus*) embryos collected from the Great Lakes, basal corticosterone concentrations (2-8 ng/ml) were negatively correlated with PCDD/PCDF, total PCBs and non-ortho PCBs (Lorenzen et al. 1999).

Amphibians and herring gulls from the Great Lakes and St. Lawrence River basin have exhibited lower basal corticosterone correlated with organochlorine exposure. Tree swallow nestlings collected from the St. Lawrence, Grasse and Raquette River contained mean total PCB concentrations of 69.13 μg/g ww, non-ortho PCBs of 0.40 μg/g ww, PCDD levels of 79.51 ng/kg ww, and PCDF levels of 128.50 ng/kg ww (see Chapter 2). We examined the relationship between corticosterone levels in tree swallows (*Tachycineta bicolor*) exposed to a gradient of environmental contamination.

Tree swallows are aerial insectivores which feed in close proximity to their nests (Robertson et al. 1992), making the nestlings indicative of local sediment contamination (Bishop et al. 1995). They also utilize a cavity nesting habitat, but cannot excavate their own nest hole. They readily use nest boxes supplied for them making it relatively easy to approach the nestlings within the nest box without much disturbance. We tested the hypothesis in 1999 and 2000 that a correlation exists between corticosterone and contaminant concentrations in these birds. Since diurnal rhythms in corticosteroids occur in other vertebrates (Leboulenger et al. 1982; Wingfield et al. 1984; Westerhof et al. 1994) and this is the first study of corticosterone response in tree swallows, the second hypothesis we tested in 1999 was that levels of corticosterone would exhibit a diurnal rhythm. We also tested the hypothesis that reproductive success of tree swallows would be significantly lower in contaminated sites compared to reference sites.

3.2. Methods and Materials

3.2.1. Study sites

Tree swallow nesting boxes were erected and sampled along the St. Lawrence River in Canada and USA in an Area of Concern (AOC), and three sites outside the AOC in 1999. The highly contaminated AOC sites were Turtle Creek (44°59N, 74° 44'W), Grasse River North (44°59N, 74° 38'W), Grasse River South (44°59N, 74° 38'W), and the two less contaminated sites were Cornwall Island (44°59N, 74° 44'W), and Cornwall Sewage Treatment plant (45°52N, 74° 42'W) for 1999. In 2000, an additional AOC site was included (Raquette River (44°59N, 74° 42'W)). The three non-AOCs included Hoasic Creek (44°57N, 57° 10'W), Upper Canada Bird Sanctuary (44°59N, 75° 01W), and Cooper Marsh (45°07N, 74° 31W). Upper Canada Bird Sanctuary was excluded in 2000. These sites were chosen to provide a gradient of contamination.

3.2.2. Tissue collection and corticosterone analysis

One 16 day old tree swallow was collected from each nest for biomarker analysis. Nestlings were collected at the AOC sites as follows: Turtle Creek (n=7 in 1999, n=10 in

2000), Grasse River North (n=2 in 1999, n=3 in 2000), Grasse River South (n=3 in 1999, n=4 in 2000), Cornwall Island (n=10 in 1999, n=7 in 2000), Sewage Treatment plant (n=8 in 1999, n=10 in 2000), and Raquette River (n=10 in 2000). Sample size were as follows in the three non-AOC sites: Hoasic Creek (n=3 in 1999, n=10 in 2000), Upper Canada Bird Sanctuary (n=7 in 1999), and Cooper Marsh (n=8 in 1999, n=10 in 2000).

Chicks were collected between June 1 and July 15, 1999, and June 15 and July 20 2000. Each nestling was removed from the nest box and immediately blood sampled by jugular venipuncture with a 1/2 cc insulin syringe (100 μl) within 1 min of retrieval for basal corticosterone levels (Holberton et al. 1996). The nestling was then placed in a dark box for 10 min, blood sampled again as a measure of stress corticosterone levels, and in 1999 placed in the dark box for an additional 10 min and sampled again to establish a corticosterone peak (Schwabl et al. 1980; Wingfield et al. 1984; 1999).

Each chick was then weighed and euthanized by CO_2 and stored at -5°C within one hour of collection and then archived at -20°C until analyzed for organochlorine contaminants. The whole body (minus feet, beak and feathers) were analyzed for organochlorine pesticides (OC), polychlorinated dibenzodioxins (PCDD), polychlorinated dibenzofurans (PCDF), non-ortho-polychlorinated biphenyls (noPCB), PCBs, and mercury analysis (see Chapter 2 for details on analytical methods).

Plasma was separated by centrifuging the blood samples at 2500 rpm in a mini-centrifuge, and plasma was stored at –20°C until analyzed for corticosterone using a commercially available ^{125}I radioimmunoassay kit (Immunochem Double Antibody, ICN, Costa Mesa, CA) (Lorenzen et al. 1999). Briefly, the procedure involved diluting plasma

(1:20) with steroid diluent in 10 x 75 mm tubes for radioimmunoassay (RIA) for a total volume of 100 µl. Non specific binding (NSB) tubes, 0 calibrator, and standards (25 ng/ml to 1000 ng/ml) were included in the assay. 200 µl I^{125}-corticosterone was added to the tubes, and 200 µl anti-corticosterone was added to all tubes except the NSB tubes. Samples were then vortexed and incubated at room temperature for 2 h. Following incubation, 500 µl precipitant solution was added to all samples and the tubes centrifuged at 2500 rpm for 15 minutes. Samples were aspirated and the tubes containing the pellet were counted using a Gamma counter. Prior to analysis, plasma samples were extracted and resuspended in distilled water and dichloromethane, and placed on dry ice to obtain the organic phase. Following drying under nitrogen at 40°C, extracts were reconstituted in phosphate buffer (pH 7.0) and aliquots were then used in duplicate. Intra and interassay coefficients of variation were 8-10%, and 11-14%, respectively, and extraction efficiency ranged from 82 to 96%. Cross reactivity, as reported by ICN for other steroids ranged from <0.01 for estradiol, cholesterol to 0.34 for deoxycorticosterone. The detection limit was 2.5 ng/ml, and plasma dilutions (in phosphate buffer) of 1:20 were determined to be optimal for tree swallow nestlings.

Reproductive success

Each nest box was 8-10 m from its nearest neighbor and located in open areas within 100 m of open water. Entrances to all nest boxes faced south to avoid prevailing wind. Nest predators were deterred by applying axle grease to nest box poles prior to the start of breeding season. Nest boxes were made of pine, unpainted on the inside, and uniform in size (approximately 12 x 12 x 20 cm). Nest boxes were visited every second day upon initiation of nest building until first egg was laid. Nests were then visited every day until last egg was laid to determine hatching date. Nests were revisited ten to fifteen days after the last egg was laid until hatching occurred to determine day 16 of chicks. Since we found tree swallows would prematurely fledge if nest boxes were opened after 16 days, the assumption was that number of chicks in nest box at 16 days represents number fledged by 19 to 21 days when tree swallows normally fledge (Robertson et al. 1992). Clutch size, hatching success (number of chicks hatched/number of eggs laid), and fledging success (number of chicks fledged/number of chicks hatched) were recorded. Age of nesting female (after second year (ASY) or second year (SY)) based on plumage criteria (Robertson et al. 1992) was also recorded.

3.2.3. Statistical analysis

All chemical and biological measurement data were tested for homogeneity of variance and normal distribution. Two-way ANOVA was performed on basal corticosterone and 10 min stress corticosterone levels (10 min stress - basal) to evaluate differences between morning (8:00 to 11:00 am) and afternoon (11:01 to 4:00 pm) blood sampling. Nested ANOVA or Kruskall-Wallis tests were performed on log transformed basal corticosterone, log transformed stress corticosterone, and reproductive parameters (body weight (g), number of eggs laid, % hatch, % fledge) to determine differences among sites. Kruskall-Wallis tests were performed on reproductive parameters to determine differences among sites. Significant differences were determined using Tukey's multiple comparison procedure. Correlations between contaminant and corticosterone levels were determined using Spearman Rank correlation. In 1999 correlations were made between 1 pool of 3 chicks per site for all PCDD, PCDF congeners and total mercury with 1 pool of 3 chicks per site for basal and stress corticosterone. In 2000 correlations were made between 2 pools of 3 chicks per site (except Grasse River N and S, n=1) for all PCDD, PCDF congeners and total mercury with 2 pools of 3 chicks per site (except Grasse River N and S, n=1) for basal and stress corticosterone. In 1999 and 2000 correlations were made between individual total PCB and organochlorine congeners with individual basal and stress corticosterone. A significance value of p=0.05 was considered statistically significant in all analysis. Bonferonni adjustments were included on p values for all correlations (Sokol and Rohlf 1991).

Two-way ANOVA indicated that some significant differences existed for corticosterone levels between the two years sampled. Therefore, data was analyzed on the basis of comparison of sites for individual years (1999 and 2000). In 2000, all blood sampling for corticosterone occurred in the morning (8:00 to 11:00 am) to minimize effect of diurnal rhythms. All significant results for correlations were verified visually by scatterplot. All statistical analysis was conducted with Statistica version 5.0 software.

3.2.4. Contaminant Analysis

16 day old chicks were analysed for non-ortho PCBs, total PCBs, dioxins, furans and mercury. For methods and detection limits of analysis see Chapter 2.

3.3. Results

3.3.1. Contaminant concentration and Plasma Corticosterone - 1999

Significant spatial differences were found in contaminant concentrations of 16 day old tree swallow nestlings. Total PCBs ranged from 0.03 µg/g ww (Hoasic Creek) to 31 μg/g ww (Grasse River S); organochlorine pesticides ranged from 0.01 μg/g ww (Hoasic Creek) to 0.13 μg/g ww (Cornwall Island); total non-ortho PCBs ranged from 297 ng/kg ww (Hoasic Creek) to 159, 640 ng/kg ww (Turtle Creek); total PCDD ranged from 5.4 ng/kg ww (Hoasic Creek) to 79.5 ng/kg ww (Grasse River N); and total PCDF ranged from 4.8 ng/kg ww (Hoasic Creek) to 94.1 ng/kg ww (Turtle Creek) (see Chapter 2).

There were no significant differences among sites in 1999 for morning and afternoon samplings of basal and 10 min stress corticosterone levels. However, there were trends indicating higher levels of basal corticosterone in the morning hours compared to afternoon for every site sampled in both times of the day (Table 3.1). Therefore all samples in 2000 were consistently taken between 8:00 am and 11:00 am.

Nested ANOVA revealed that there were no significant differences among exposure groups (contaminated vs non-contaminated), but there were significantly higher mean basal corticosterone levels at Hoasic Creek ($F=3.8$, $p=0.006$) compared to all other sites (Figure 3.1a) (Appendix Table 7). Chicks from Grasse River N exhibited the lowest basal corticosterone levels (Figure 3.1a). Basal corticosterone ranged from 4 ng/ml (Grasse River N) to 37 ng/ml (Hoasic Creek), and was negatively correlated to PCDF ($p=0.01$, $r=0.82$) (Figure 3.2a). With a bonferonni adjustment ($p=0.007$) there is no significant correlation between corticosterone and PCDF.

There were no significant differences among sites, or between exposure groups for stress corticosterone at the ten minute stress interval ($F=1.6$, $p=0.183$) (Figure 3.3a). The range for corticosterone at 10 min stress sampling was 14 ng/ml (Cornwall Island) to 47 ng/ml (Grasse River N) (Appendix Table 7). Corticosterone concentration at 10 minute stress sampling was positively correlated with total PCDD (p=0.04, r=0.74) (Figure 3.4a), and 1,2,3,7,8 PeCDD (p=0.0008, r=0.91) (Figure 3.4b). After correction with a bonferonni adjustment only the correlation between stress corticosterone and PeCDD was significantly positive. There were no correlations between corticosterone at any time point with body weight, % lipid in chicks, or with date sampled.

Reproductive success - 1999

Body weight of chicks at 16 days old did not differ among sites (p=0.67, F=0.69) (Appendix Table 7). Although the number of eggs laid did not differ among sites (p=0.44, H=6.88), Hoasic Creek had the lowest average clutch size (n=4.8). Percent hatch did not differ among sites (p=0.06, H=14.23). However Grasse River S had the lowest percent hatch (67%) of all the sites. Percent fledging did not differ among sites (p=0.57, H=5.71) (Table 3.2). There were no significant differences among sites for number of second year (SY) or after second year (ASY) females nesting (p=0.31, H=8.31).

3.3.2 Contaminant Concentration and Plasma Corticosterone - 2000

Significant spatial differences were found in contaminant concentrations of 16 day old tree swallow nestlings. Total PCBs ranged from 0.01 μg/g ww (Hoasic Creek) to 69.13 μg/g ww (Grasse River S); organochlorine pesticides ranged from 0.02 μg/g ww (Hoasic Creek) to 0.06 μg/g ww (Cornwall Island); total non-ortho PCBs ranged from 312 ng/kg ww (Hoasic Creek) to 404, 605 ng/kg ww (Turtle Creek); total PCDD ranged from 6.60 ng/kg ww (Hoasic Creek) to 32.40 ng/kg ww (Grasse River N); and total PCDF ranged from 5.00 ng/kg ww (Hoasic Creek) to 128.50 ng/kg ww (Turtle Creek) (see Chapter 2).

Body weights in 2000 were not significantly different among sites (p=0.11, F=1.7) (Appendix Table 8). Grasse River N (19.94 g), Hoasic Creek (20.52 g), and Raquette River (20.68 g) were among the lowest weights, and chicks from STP had the highest weights (22.89 g). The number of eggs among sites did not differ (p=0.69,H=4.73). Percent hatch was not significantly different among sites (p=0.57, H=5.68). However, the lowest hatching success was found at Grasse River S (67%). Fledging success was also not variable among sites (p=0.63, H=5.24) (Table 3.6). There were no significant differences among sites for number of second year (SY) or after second year (ASY) females nesting (p=0.08, H=12.7).

Table 3.1. Two-way ANOVA results of log transformed basal[b] (ng/ml) and log transformed stress corticosterone[a] (ng/ml) levels in the morning (am) (sample size in parenthesis) and afternoon (pm) (sample size in parenthesis).

<u>1999</u>

Site	basal corticosterone		p value	stress corticosterone		p value
	am (n)	pm (n)		am	pm	
Cornwall Island	8.63 (6)	3.98 (5)	0.22	5.89	8.32	0.55
Turtle Creek	7.94 (4)	3.16 (2)	0.58	13.81	12.99	0.28
Hoasic Creek	37.40 (3)	-	-	16.05	-	-
Cooper Marsh	5.84 (7)	6.31 (1)	0.98	17.01	15.79	0.17
Sewage Treat. P	6.31 (6)	5.01 (2)	0.49	7.94	3.23	0.63
UC Bird Sanc.	19.95 (6)	15.84 (1)	0.07	39.81	60.25	0.43
Grasse River N	5.88 (1)	1.99 (1)	-	1.90	3.63	-
Grasse River S	13.80 (1)	6.31 (1)	-	50.11	7.34	-

stress corticosterone[a] -10 minute stress corticosterone minus basal corticosterone
basal corticosterone[b] - blood sample retrieved within 1 min of collecting chick from nest box.
- not available

Table 3.2 Mean and standard deviation of number of eggs laid, % hatch, and % fledge from tree swallow nest boxes along the shores of the St. Lawrence River.

1999

Site(*)	Number of eggs[a]		%Hatch[b]		%Fledge[c]	
	Mean	SD	Mean	SD	Mean	SD
Sewage Treatment P(c)	6.0	1.0	.98	.05	1.0	0.00
Cooper Marsh (r)	6.3	0.6	.94	.08	1.0	0.00
Upper Canada Bird Sanc(c)	5.4	1.0	.99	.04	0.8	0.38
Cornwall Island (c)	5.7	1.3	.95	.08	09	0.25
Turtle Creek (c)	5.6	0.9	.98	.05	1.0	0.00
Hoasic Creek (r)	5.3	1.2	.98	.06	0.9	0.14
Grasse River N (c)	4.3	2.1	.94	.10	1.0	0.00
Grasse River S (c)	4.5	2.4	.67	.45	1.0	0.00

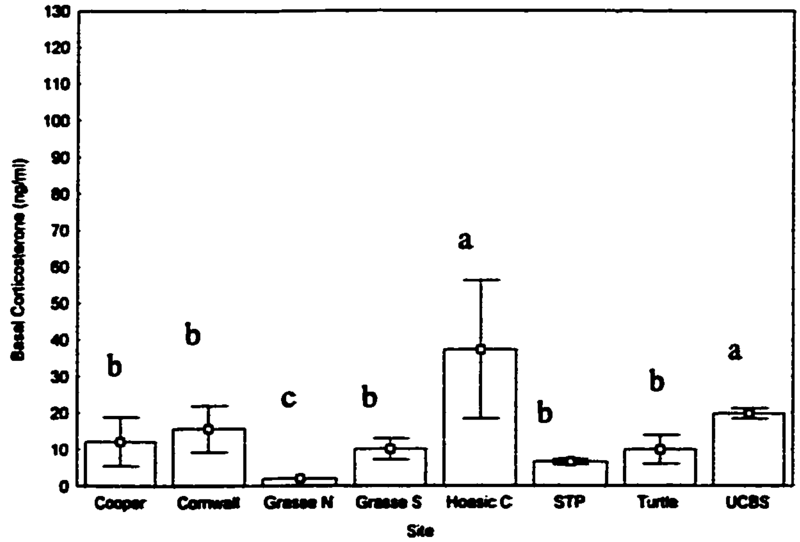
eggs[a] Number of eggs laid
%hatch[b] Number of eggs hatch/# eggs laid
%fledge[c] Number of chicks that fledge the nest (after 16d)
site (*) (c) = AOC, (r) = reference

Table 3.3. Table 3.2 Mean and standard deviation of number of eggs laid, % hatch, and % fledge from tree swallow nest boxes along the shores of the St. Lawrence River.

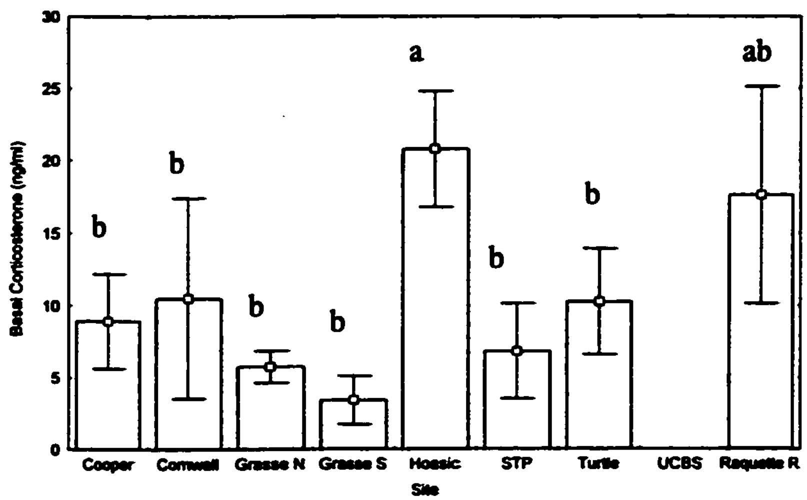
2000

SITE (*)	EGG[a] Mean	EGG[a] SD	%HATCH[b] Mean	%HATCH[b] SD	%FLEDGE[c] Mean	%FLEDGE[c] SD
Sewage Treat. Plant (c)	5.6	0.5	0.85	0.21	0.9	0.1
Cooper Marsh (r)	5.4	1.0	0.92	0.10	1.0	0.0
Cornwall Island (c)	5.0	2.0	0.87	0.33	0.9	0.1
Turtle Creek (c)	5.7	1.1	0.82	0.20	0.9	0.1
Hoasic Creek (r)	5.0	1.1	0.95	0.08	0.9	0.1
Grasse River N (c)	5.2	0.9	0.94	0.12	1.0	0.0
Grasse River S (c)	5.5	1.7	0.68	0.47	1.0	0.0
Raquette River (c)	5.7	0.8	0.93	0.14	0.9	0.0

eggs[a] Number of eggs laid
%hatch[b] Number of eggs hatch/# eggs laid
%fledge[c] Number of chicks that fledge the nest (after 16d)
site (*) (c) = AOC, (r) = reference



[A]



[B]

Figure 3.1. Basal corticosterone (ng/ml) (± SE) in 16 d old tree swallow plasma collected from the St. Lawrence river in [A] 1999 and [B] 2000. In both years there is a significant difference among sites donated by a,b,c (p=0.006, F=3.8 (1999), p=0.05, F=2.12 (2000)).
1999 Sample sizes; Upper Canada Bird Sanctuary (7), Hoasic Creek (3), Grasse River N (2), Grasse River S (3), Turtle Creek (7), Cooper Marsh (8), Cornwall Sewage Treatment Plant (8), Cornwall Island (10).
2000 Sample sizes; Raquette River (10), Hoasic Creek (10), Grasse River N (4), Grasse River S (3), Turtle Creek (10), Cooper Marsh (10), Cornwall Sewage Treatment Plant (10), Cornwall Island (7).

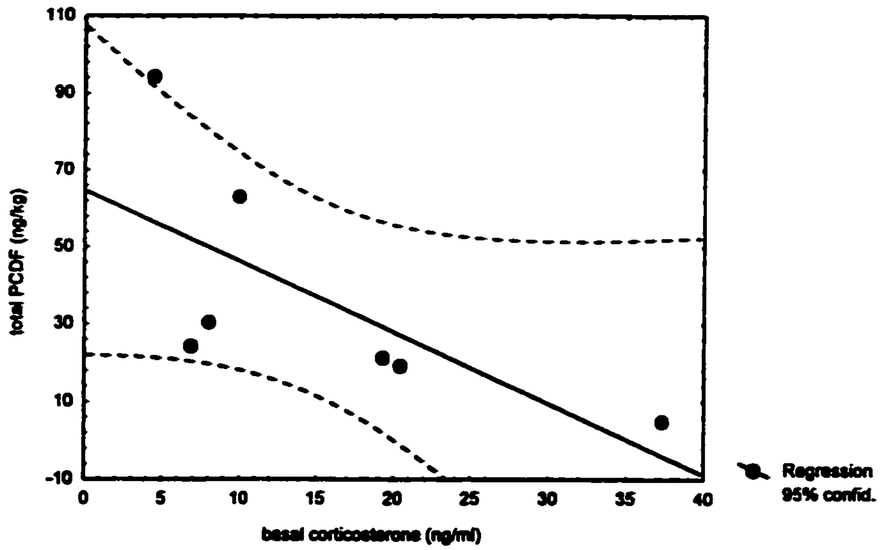
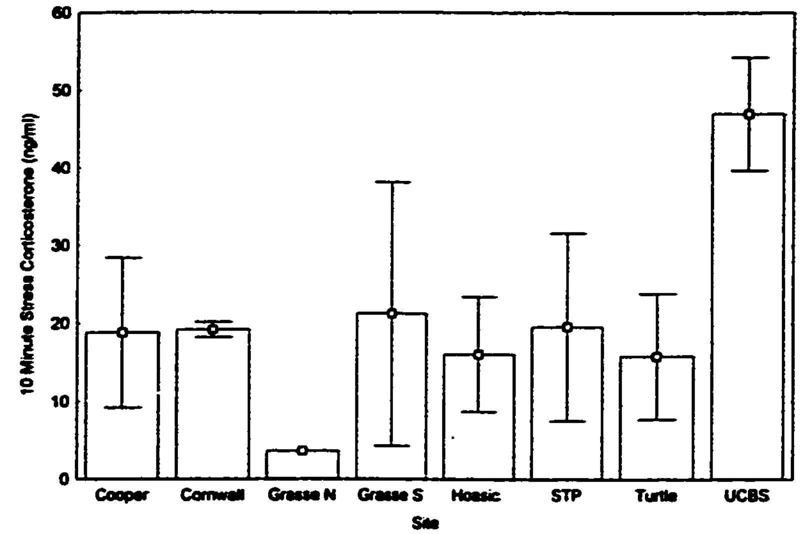
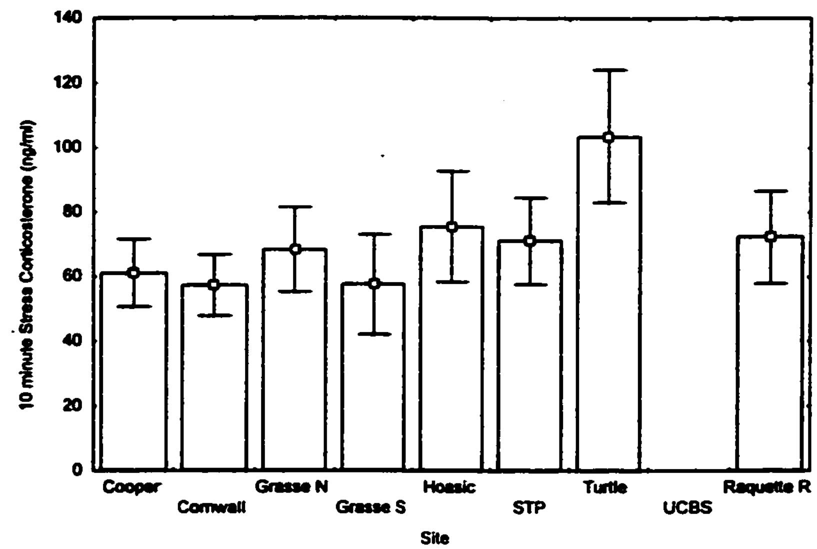


Figure 3.2a. Spearman rank correlation between total polychlorinated dibenzofuran (PCDF) and basal corticosterone (ng/ml) in 16 day old tree swallows from the St. Lawrence River (1999) ($p=0.01$, $r=0.82$).

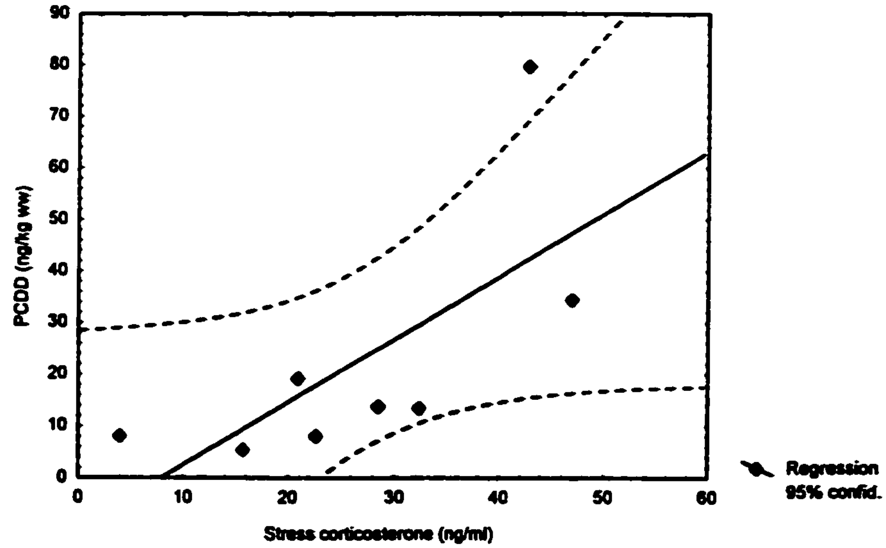


[A]

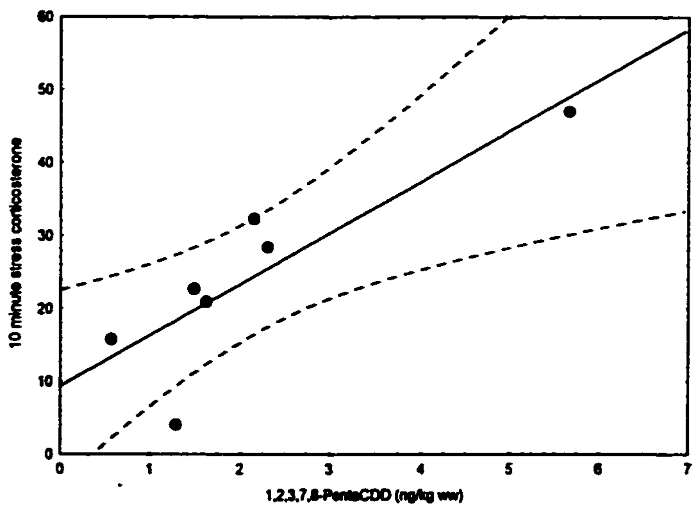


[B]

Figure 3.3. Stress corticosterone (ng/ml) (± SE) in 16 d old tree swallow plasma collected from the St. Lawrence River in [A] 1999 and [B] 2000.



[A]



[B]

Figure 3.4. Spearman rank correlation between [A] PCDD (ng/kg ww) and 10 min stress corticosterone (ng/ml) in 16 d old tree swallow nestlings from the St. Lawrence River (1999), p=0.04, r=0.71); and [B] 1,2,3,7,8 PeCDD (ng/kg ww) and 10 min stress corticosterone (ng/ml) in 16 d old tree swallow nestlings from the St. Lawrence River (1999), p=0.0008, r=0.91).Stress corticosterone is 10 minute corticosterone minus basal corticosterone.

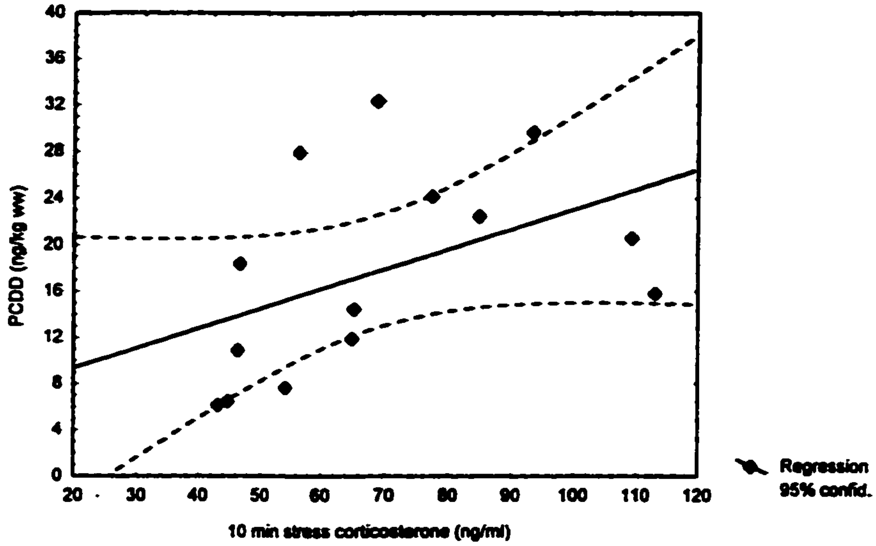


Figure 3.5. Spearman rank correlation between PCDD (ng/kg ww) and 10 min stress corticosterone (ng/ml) in 16 d old tree swallow nestlings from the St. Lawrence River, (2000), p=0.01, r=0.65).

Two pools of 3 chicks/site (except Grasse River N and Grasse River S, n= 1 pool).

10 minute stress corticosterone = stress corticosterone - basal corticosterone

3.4. Discussion

This study found a trend, but no significant differences in diurnal variation of corticostorone concentrations in tree swallow nestlings. We found few significant differences in 10 minute post stress corticosterone concentrations among sites in 1999 and 2000, but did find significant differences among sites for basal corticosterone. Additionally, we found a positive correlation between corticosterone levels (10 minutes post stress) and polychlorinated dibenzodioxin (PCDD) in both years of the study, and a negative correlation between basal corticosterone and total polychlorinated dibenzofuran (PCDF) in 1999.

Corticosterone is involved in the initiation of gluconeogenesis, required for energetically demanding tasks. Thus, levels of these hormones would be expected to exhibit diurnal rhythms in passerines that are most active during the first part of the day. This study showed that generally, levels of basal corticosterone in tree swallow nestlings were higher in the morning than in the afternoon at most sites sampled. Seasonal and diurnal rhythms also occur in a number of other species including amphibians (Leboulenger et al. 1982) and birds (Wingfield 1984; Westerhof et al. 1994) with higher circulating corticosterone in the morning for birds, and evening for frogs. Levels of basal corticosterone in tree swallows ranged from 4 to 20 ng/ml. This is similar to ranges of basal corticosterone for other species such as herring gull embryos from the Great Lakes (2 to 12 ng/ml) (Lorenzen et al. 1999), premigratory Gray catbird (*Dumetella carolinensis*) (20 to 30 ng/ml), migratory yellow-rumped warblers (*Dendroica coronata*) (30 to 32 ng/ml) (Holberton et al. 1996) and black-throated sparrow (*Amphispiza bilineata*) (4-6 ng/ml) (Wingfield et al. 1999). In tree swallows corticosterone after 10 minute stress ranged from 14 ng/ml to 99 ng/ml. Stress corticosterone after 30 minutes capture rose to 70 ng/ml in Gray catbirds, and 35 ng/ml in Black-throated sparrows. Maximum corticosterone levels in Gambel's white-crowned sparrow (*Zonotrichia leucophyris gambelii*) reached 48 ng/ml during early light phase of long days (Breuner et al. 1999). Although caution should also be taken when comparing corticosterone levels between different studies, tree swallows appear to exhibit corticosterone concentrations similar to other birds tested. Numerous factors may account for the variation, including analytical methods, blood sampling methods, diurnal rhythms, seasonal rhythms relating to migration and fat storage, age, contaminants, and weather impacts.

In addition to natural rhythms that control corticosterone concentrations, contaminant levels may also influence levels of corticosterone in the morning vs afternoon. For example, dioxin treated rats exposed to 50 μg/g body weight TCDD showed an increase in corticosterone in the light phase (AM), but caused a decrease of 40% in the late light phase (PM). The AM stimulation of steroidogenesis by TCDD may result from an increase in cholesterol transfer. The inhibition of cholesterol side chain cleavage resulting from TCDD treatment, may be more limiting for corticosterone synthesis when cholesterol transfer is activated, as for peak PM secretion (DiBartolomeis et al. 1987). Since cholesterol is the precursor to corticosterone, an increase in cholesterol in the morning could also result in an increase of corticsterone. This may also offer a partial explanation for sensitivity of the hypothalamo-pituitary-adrenal (HPA) axis in early light phase sampling and contaminant exposure, and should be taken into account when conducting toxicological field studies.

Stress induced corticosterone levels in the current study were positively correlated with total PCDDs, and 1,2,3,7,8 PeCDD in 1999 and with total PCDDs in 2000. Because corticosterone is a glucocorticoid, it mobilizes the production of glucose as an energy source in animals during food deprivation. Corticosterone may elevate circulating levels of corticosterone in response to hypoglycemia because of a disruption in gluconeogenesis. This may be a partial explanation for the trend of a positive correlation between organochlorine exposure and corticosterone concentrations found in the tree swallow nestlings. Although few studies have found an increase in corticosterone with levels of dioxin, TCDD does have a profound effect on glucose metabolism causing a decrease in glucose levels (Gorski et al. 1988a). Since corticosterone is a key hormone in the initiation of gluconeogenesis and regulation of glucose homeostasis, it is probable that elevated corticosterone levels in animals exposed to TCDD are a response to "metabolic stress" related to glucose metabolism. It has been further proposed that this interaction causes corticosterone production to increase in response to low glucose levels (Gorski et al. 1988b; Weber et al. 1991).

Corticosterone is stimulated by the release of adrenocorticotrophic hormone (ACTH) in the avian system. Once into circulation, ACTH interacts with membrane-bound receptors in adrenal cells to activate adenylate cyclase which generates cAMP and protein kinases (Norris 1997). Protein kinases then initiate the manufacturing of cholesterol which is cleaved by CYP1A1 enzymes to produce pregnenolone, the precursor to corticosterone. Rats treated with a single dose of TCDD caused ACTH and corticosterone to increase in past studies (Weber et al. 1991). The impact of TCDD on ACTH concentrations may also explain differences seen in corticosterone levels.

Basal levels of corticosterone were higher at the reference site, Hoasic Creek, compared to most other sites in both years of this study. One possible explanation for this could be that samples taken in 1999 were in the morning for Hoasic Creek, compared to sampling in the morning and afternoon hours for the rest of the sites. Corticosterone levels are expected to be higher in the morning prior to feeding and could have contributed to higher corticosterone levels. In 2000, all samples at all sites were taken in the morning to account for diurnal rhythms. However, corticosterone levels at Hoasic Creek were still higher. Basal levels of corticosterone were also negatively correlated with PCDF in 1999. A reduction in basal corticosterone may be the result of depressed enzyme activity responsible for conversion of cholesterol to corticosterone. Previous studies have indicated lower resting levels of corticosterone and cortisol in animals exposed to environmental contaminants. Corticosterone levels in herring gull eggs collected from the Great Lakes were negatively correlated to dioxins, furans, total PCBs and non-ortho PCBs (Lorenzen et al. 1999). Similarly, inverse relationships were found between two intermediary enzymes involved in gluconeogenesis, phosphoenolpyruvate carboxykinase and malic enzyme when regressed against PCDD/PCDF. Teleost fish exposed to mixtures of PCBs, PAHs (polyaromatic hydrocarbons), mercury and other metals showed a decreased ability to elevate cortisol in response to an acute stress test (Hontela 1997).

Biological factors such as age, weight, body condition, and feeding rate have the potential to confound results by being correlated at the same time to the HPA axis activity and to contamination status. For example, studies with mammals suggest that age is associated with loss of sensitivity of adrenal gland to ACTH (Gendron et al. 1997).

However, age, body weight and % lipid were not significantly correlated with plasma corticosterone in this study.

No indications were found of abnormal body weights in tree swallow nestlings from the St. Lawrence River basin. Bishop et al. (1999) found no correlation between organochlorine contamination and tree swallow nestling body weight from the St. Lawrence River basin. Similarly, McCarty and Secord (1999) found no change in growth rate of tree swallows exposed to high levels of PCBs (62.2 μg/g ww). Alternatively, other studies have found impaired growth in birds exposed to environmental contaminants (Fox 1993). "Wasting syndrome" has been associated with loss of pectoral muscle in Forster's terns (*Sterna forsteri*) environmentally exposed to PCBs (5.4 - 9.5 μg/g ww) (Harris and Osborn 1981). It is possible that prolonged elevated corticosterone levels would result in wasting since corticosterone utilizes protein for gluconeogenesis but no evidence of this was found in this study.

Hatching success was not significantly different among sites. However, Grasse River S, located within the vicinity of ALCOA and Reynolds Metals had the lowest percent hatch (67% in 1999 and 2000) of all the sites. These rates are even lower than tree swallow eggs from the Hudson River contaminated with PCB concentrations up to 43 μg/g, where hatching success was 64 to 79% (Secord and McCarty 1997). All other sites in this study were above 82%, which are similar to mean rates of 86% reported in a review of seven non contaminated North American sites where hatching was defined by at least one egg hatched (Robertson et al. 1992).

Fledging success of tree swallow chicks exceeded 87% at all sites along the St. Lawrence river. Rates above 75% are comparable to those of other tree swallow populations in non-contaminated sites (Robertson et al. 1992). In contrast, at contaminated sites on the Hudson River, fledging rates were 51-74% (Secord and McCarty 1997). Mean rates at Cornwall Island in 1994 were low at a rate of 58% (Bishop et al. 1999). Lower than average reproductive success may be associated with numerous factors including subadult females utilizing next boxes. Second year females have lower fledgling and hatching success compared to after second year females (Robertson et al. 1992; Secord and McCarty 1997; Bishop et al. 1999).

Grasse River S also had the lowest clutch size in both years of the study. However, there were no significant differences among sites for number of nesting second year females so this is unlikely to be a factor. Nontheless boxes were erected in 1999 at Grasse River S, which may account for less colonization of tree swallows and result in low reproductive success. Both clutch size (Hussell and Quinney 1987) and nestling growth (Quinney et al. 1986) have also been linked to differences in insect abundance, and nestling size has been shown to be an indicator of environmental stress (Zach and Mayoh 1984). The lack of variation between sites in body weight and reproductive success, imply an absence of extrinsic and intrinsic factors affecting these parameters.

3.5 Summary and Conclusions

A gradient of contamination existed in tree swallow nestlings among sites along the St. Lawrence River basin. Concentrations of total PCBs, non-ortho PCBs, and furans in 1999 and 2000 were highest in tree swallow nestlings from Turtle Creek, Grasse River S, and Grasse River N. The highest levels of dioxins were found in chicks from Grasse River N in 1999 (see Chapter 2).

Basal corticosterone concentrations of individual nestlings were highest at one of the cleanest reference sites of the study, Hoasic Creek. Basal corticosterone levels were negatively correlated with furans among sites in 1999. Stress induced corticosterone levels were positively correlated with dioxins among sites in 1999 and 2000. In addition to the role that corticosterone plays in protein, lipid and carbohydrate metabolism, it also has immunosuppressive effects. High glucocorticoids reduce the number of antibody producing cells and circulating lymphocytes (Ribelin 1984), which may lead to an increased incidence of infections by bacteria, protozoan in animals with hyper activity of corticosterone or cortisol. Another implication of elevated levels of corticosterone is its possible negative effects on growth and weight gain in birds. This steroid induces breakdown of both protein and nucleic acid and decreases protein synthesis to increase the amino acid substrate for gluconeogenesis. Long term exposure to PCDD may lead to increase wasting, characterized by weight loss in young chicks. Though little significant differences in reproduction and body weight have been found in this study, loss of muscle may be manifested in impeded migratory function in fledged nestlings. Since the pathway for glucocorticoids is similar to that of testosterone and estrogens, research examining the possible interference of other critical hormone processes is warranted.

Chapter 4

4.0 VITAMIN A CONCENTRATIONS IN TREE SWALLOWS (*TACHYCINETA BICOLOR*) EXPOSED TO A GRADIENT OF ENVIRONMENTAL CONTAMINATION.

4.1 Introduction

Vitamin A is a fat soluble essential vitamin which largely occurs in nature as retinol. Retinol can be enzymatically or chemically modified to become a polar metabolite such as retinoic acid, or an ester as in retinyl palmitate, a form that accounts for over 95% of body stores (Green and Green 1994). The mobilization of hepatic retinoids is partially controlled by formation of the retinol:retinol-binding-protein (RBP) complex which in turn binds with high affinity to a second transport protein called transthyretin (TTR). Together, RBP and TTR regulate the concentration of circulating vitamin A, so that levels are constant over a wide range of dietary intakes (Green and Green 1994). This system ensures that retinol is available to cells for conversion to retinoic acid, which regulates growth and development.

Despite the controlled regulation of vitamin A, contaminant exposure can disrupt vitamin A dynamics by one of two methods (Simms et al. 2000). The first mechanism is based on the ability of polychlorinated biphenyls (PCBs), polychlorinated dibenzo-dioxins (PCDDs), and polychlorinated dibenzo-furans (PCDFs) to bind and activate the aryl hydrocarbon receptor (Van den Berg 1998). The resultant complex binds to DNA in the nucleus and alters transcription of specific proteins which control vitamin A storage and catabolism. In addition, some hydroxy metabolites of polychlorinated biphenyls (PCBs), polychlorinated dibenzodioxins (PCDDs), polychlorinated dibenzofurans

(PCDFs) bind to the TTR and displace thyroxine, resulting in the filtration of vitamin A in the kidney (Green and Green 1994).

A number of abnormalities are associated with depressed levels of vitamin A in avian species. Delayed growth, and ocular discharge have all been associated with chicks on low vitamin A diets. Reduced reproductive capacity was noted in mature leghorn hens (Bermudez et al. 1993) and chickens (Spear et al. 1986) fed to vitamin A deficient diets.

Vitamin A deficiency in wild birds from contaminated sites along the Great Lakes was first noted in the mid 1970s when Fox (1993) compared levels of hepatic retinyl palmitate and hepatic organochlorine (OC) residues in herring gulls from eight locations and a coastal reference colony between the years of 1974 to 1993. Most contaminant residues decreased over time (Government of Canada 1991; Hebert et al. 1994), while retinyl palmitate (storage form) levels increased significantly. Similarly, Spear et al. (1986) also noted that levels of hepatic retinol and retinyl palmitate were significantly lower in Great Lakes gulls (*Larus argentatus*) compared to New Brunswick gulls. Bishop et al. (1999) found negative trends between liver EROD and liver retinyl palmitate ($R=-0.74$, $p=0.06$) and kidney retinol ($R=-0.72$, $p=0.07$) levels in tree swallow nestlings (*Tachycineta bicolor*) collected from the St. Lawrence River. Boily et al. (1994) found retinyl palmitate in the eggs of Great blue herons (*Ardea herodias*) to be negatively correlated with PCB congeners 105 and 118.

A positive relationship between the molar ratio of retinol:retinyl palmitate and environmental contaminants has also been established in herring gull eggs collected during early incubation from seven colonies in Lakes Huron and Ontario (Spear et al. 1990). The authors found that the molar ratio of retinol to retinyl palmitate was positively correlated with 2,3,7,8-TCDD, and with the sum of the PCDD and PCDF toxic equivalent factors (TEQs). Elliott et al. (2001) found a positive relationship between liver retinol and total PCBs in Osprey chicks (*Pandion haliaetus*). Although it has been established that dietary exposure to organochlorine contaminants may alter vitamin A dynamics, it is not clear whether these effects lead to hypo- or hyper-vitaminosis A.

In the present study, 16 day old tree swallows were collected from sites representing a gradient of organochlorine contamination along the St. Lawrence River, to determine if there is a relationship between retinol and retinyl palmitate levels as a function of organochlorine contaminant levels. Tree swallow nestlings collected from the St. Lawrence, Grasse and Raquette River contained mean total PCB concentrations of 69.13 μg/g ww, non-ortho PCBs of 0.40 μg/g ww, PCDD levels of 79.51 ng/kg ww, and PCDF levels of 128.50 ng/kg ww (see Chapter 2). The hypothesis is that there will be a negative correlation between hepatic vitamin A concentration and organochlorine contamination in tree swallow nestlings.

4.2 Materials and Methods

4.2.1 Study Sites

Tree swallow nesting boxes were erected and sampled along the St. Lawrence River in Canada and USA in an Area of Concern (AOC), and three sites outside the AOC in 1999. The highly contaminated AOC sites were Turtle Creek (44°59N, 74° 44'W), Grasse River North (44°59N, 74° 38'W), Grasse River South (44°59N, 74° 38'W), and the two less contaminated sites were Cornwall Island (44°59N, 74° 44'W), and Cornwall

Sewage Treatment plant (45°52N, 74° 42'W) for 1999. In 2000, an additional AOC site was included (Raquette River (44°59N, 74° 42'W)). The three non-AOCs included Hoasic Creek (44°57N, 57° 10'W), Upper Canada Bird Sanctuary (44°59N, 75° 01W), and Cooper Marsh (45°07N, 74° 31W). Upper Canada Bird Sanctuary was excluded in 2000. These sites were chosen to provide a gradient of contamination.

4.2.2 Tissue collection

One 16 day old tree swallow nestling was collected from each occupied box (n= 10 boxes) from each of the nine sites. Mass of birds was measured in the field with a 50 (±0.5)

g pesola (see Chapter 2).. Birds were asphyxiated with CO_2 and brought to the lab for dissections. Travel time ranged from 5 to 25 minutes. Liver and kidney were removed, weighed (to the nearest 0.01g), and placed in liquid nitrogen for storage until analysed for vitamin A (retinol and retinyl palmitate).

In the second year of the study, insect boluses (insect food bundle brought by parents and placed in the beak of chicks) were also collected by ligature from sibling chicks from the same nest not collected for analysis. This involved placing a ligature around the neck of a 8-10 day old chick and leaving the nest for ½ hour. After this time period the chick was checked for insect boluses left by the parent. The boluses were removed with sterile tweasers and immediately placed into amber vials for storage in liquid nitrogen. All boluses were collected between 07:00 to 10:00 hrs and analysed for retinoid content (retinol and β–carotene). All handling of vitamin A samples was done in minimal light to prevent degradation of samples. All handling of tree swallow nestlings was in accordance with Canadian Wildlife Service Animal Care Committee.

Retinol and retinyl palmitate in liver and kidneys

For retinol and retinyl palmitate extractions, an aliquot of about 0.3 to 0.5 g of partially thawed liver or kidney was ground with anhydrous sodium sulphate until a dry powder was obtained. A 0.2 g liver or kidney equivalent was extracted with 10 µl of dichloromethane:methanol (1:9), containing the internal standard retinyl acetate. After shaking and then centrifuging (10 min) the extract, an aliquot was filtered through a 0.45 µm filter. A 20 µl aliquot is then analysed by non-aqueous reverse-phase high-performance liquid chromotagraphy (HPLC). Retinoid compounds were detected with a flourescence detector (excitation/emission wavelengths=326/480 nm). The detection limits of retinol and retinyl palmitate are 0.4 µg/g and 1.2 µg/g. All samples were processed under minimal lighting to prevent degradation of samples. Samples were analyzed at the National Wildlife Research Centre, Quebec, by France Maisonneuve and myself.

4.4.3 Statistical analysis

All chemical and biological measurement data were tested for homogeneity of variance and normal distribution. Where these criteria were not met, we used non-parametric statistical tests (using Statistica). Nested ANOVA or Kruskall-Wallis tests were performed to determine differences among sites. Retinol and β–carotene levels in insects boluses were pooled and prevented analysis of means. Correlations between contaminant levels and vitamin A levels were determined using Spearman Rank correlation. In 1999 correlations were made between 1 pool of 3 chicks per site for all PCDD, PCDF congeners and total mercury with 1 pool of 3 chicks per site for renal and hepatic retinol, retinyl palmitate, and molar ratio of retinol to retinyl palmitate. In 2000 correlations were made between 2 pools of 3 chicks per site (except Grasse River N and S, n=1) for all PCDD, PCDF congeners and total mercury with 2 pools of 3 chicks per site (except Grasse River N and S, n=1) for renal and hepatic retinol, retinyl palmitate, and molar ratio of retinol to retinyl palmitate. In 1999 and 2000 correlations were made between individual total PCB and organochlorine congeners with individual renal and hepatic retinol, retinyl palmitate, and molar ratio of retinol to retinyl palmitate. Significant differences were found between years for the vitamin A levels leading to analysis based on separate years. A significance value of $p=0.05$ was considered statistically significant in all analysis and correlation p values were adjusted by Bonferonni (Sokol and Rohlf 1981). All statistical analysis was conducted with Statistica version 5.0 ® software.

4.3 Results

4.3.1 1999

Contaminant Trends

Significant spatial differences were found in contaminant concentrations of 16 day old tree swallow nestlings. Total PCBs ranged from 0.03 µg/g ww (Hoasic Creek) to 31 µg/g ww (Grasse River S); organochlorine pesticides ranged from 0.01 µg/g ww (Hoasic Creek) to 0.13 µg/g ww (Cornwall Island); total non-ortho PCBs ranged from 297 ng/kg ww (Hoasic Creek) to 159, 640 ng/kg ww (Turtle Creek); total PCDD ranged from 5.4

ng/kg ww (Hoasic Creek) to 79.5 ng/kg ww (Grasse River N); and total PCDF ranged from 4.8 ng/kg ww (Hoasic Creek) to 94.1 ng/kg ww (Turtle Creek) (see Chapter 2).

Liver Vitamin A

Hepatic retinol concentrations (log transformed) were not significantly different among sites (p=0.29, F=1.3) or between groups (contaminated vs non contaminated). The highest mean levels of retinol were found in chicks from Turtle Creek (7.23 μg/g ± 3.08), and the lowest levels were found at Grasse River N (3.40 μg/g ± 2.12) (Table 4.1). There were no correlations between contaminant concentrations or TEQs and hepatic retinol.

Hepatic retinyl palmitate were significantly different among sites sampled in 1999 (p=0.04, F=2.3), but not significantly different between exposure groups (non-contaminated vs contaminated). Grasse River N had the lowest mean levels (54.91 μg/g ± 30) and Cooper Marsh had the highest mean levels (146.84 μg/g ± 69) (Table 4.1). There were no correlations found between contaminant concentrations or TEQs and hepatic retinyl palmitate.

Nested ANOVA revealed no significant difference between exposure groups, but did find significant differences among sites for molar ratio of retinol to retinyl palmitate in the liver (p=0.04, H=0.04). The highest ratio was found at Grasse River S (0.19) compared to the lowest ratio found at Cooper Marsh (0.08) (Table 4.2). There were no significant correlations between contaminant concentrations or sum of TEQs and hepatic molar ratio of retinol:retinyl palmitate.

Kidney Vitamin A

Kidney retinol concentrations (log transformed) were not significantly different among exposure groups, but were significantly different among sites ($p=0.02$, $F=2.9$). Cooper Marsh chicks had the highest concentrations (1.04 μg/g) and Grasse River N had the lowest (0.35 μg/g) (Table 4.1). No correlations were found between contaminant concentrations or TEQs and renal retinol.

Nested ANOVA revealed no significant differences between exposure groups for retinyl palmitate (contaminated vs non-contaminated), but did show significant differences among sites ($p=0.04$, $F=2.3$) (Table 4.1). Turtle Creek (5.58 μg/g) and Cooper Marsh (5.21 μg/g) were significantly higher than all other sites. Grasse River N had the lowest levels (1.15 μg/g). No correlations were found between contaminant concentrations or TEQs and renal retinyl palmitate.

There were no significant differences between exposure groups, or among sites for renal retinol:retinyl palmitate ratio ($p=0.5$, $H=5.9$). The highest renal molar ratio was found at Grasse River N (1.11), followed by Grasse River S (0.77) (Table 4.2). Molar ratio of retinol:retinyl palmitate was positively correlated with total PCDD ($p=0.0280$, $r=0.7619$) (Figure 4.1a), and 1,2,3,7,8-PeCDD ($p=0.028$, $r=0.7619$) (Figure 4.2). With the highest total PCDD level excluded (Grasse River N), the molar ratio was still positively correlated with total PCDD ($p=0.013$, $r=0.857$) (Figure 4.1b), and 1,2,3,7,8-PeCDD ($p=0.036$, $r=0.785$). With a bonferonni adjustment ($p=0.007$) there is no significant correlation between molar retinol:retinyl palmitate and PCDD.

Liver and kidney

Vitamin A parameters were correlated. Liver retinyl palmitate and kidney retinol (μg/g) were positively correlated ($p=0.007$, $r=0.85$) (Figure 4.3). With a bonferonni adjustment ($p=0.007$) there is still a significant correlation between liver retinyl palmitate and kidney retinol.

4.3.2 2000

Contaminant Trends

Significant spatial differences were found in contaminant concentrations of 16 day old tree swallow nestlings. Total PCBs ranged from 0.01 μg/g ww (Hoasic Creek) to 69.13 μg/g ww (Grasse River S); organochlorine pesticides ranged from 0.02 μg/g ww (Hoasic Creek) to 0.06 μg/g ww (Cornwall Island); total non-ortho PCBs ranged from 312 ng/kg ww (Hoasic Creek) to 404, 605 ng/kg ww (Turtle Creek); total PCDD ranged from 6.60 ng/kg ww (Hoasic Creek) to 32.40 ng/kg ww (Grasse River N); and total PCDF ranged from 5.00 ng/kg ww (Hoasic Creek) to 128.50 ng/kg ww (Turtle Creek) (see Chapter 2).

Liver Vitamin A

There were no significant differences between exposure groups (contaminated vs non-contaminated) or among sites for liver retinol ($p=0.07$, $F=2.6$). Highest levels were found at Grasse River S (13.50 μg/g) and Hoasic Creek (9.46 μg/g) compared to lower levels from Cornwall Island (4.60 μg/g) (Table 4.3). There were no correlations between contaminant concentrations and hepatic retinol.

There were no significant differences between exposure groups, but there were significant differences among sites for hepatic retinyl palmitate ($p=0.002$, $F=3.7$).

Significantly higher levels were found at Raquette River (118.10 µg/g), compared to Cornwall Island, Cooper Marsh, and Turtle Creek. The lowest levels were found at Cooper Marsh (41.71 µg/g) (Table 4.3). Despite these geographic trends, there were no significant correlations between hepatic retinyl palmitate and contaminant residues or TEQs.

There were no significant differences found for hepatic retinol:retinyl palmitate ratio between exposure groups, or among sites ($p=0.62$, $H=5.3$) (Table 4.4). The highest ratio was found at Grasse River S (0.30) compared to the lowest ratio from Cornwall Sewage Treatment Plant, Raquette River, and Cooper Marsh (0.16) (Table 4.4).

Kidney Vitamin A

Nested ANOVA revealed no significant differences between exposure groups (contaminated vs non-contaminated), or among sites for kidney retinol (log transformed) ($p=0.57$, $F=0.8$). However, the highest levels were found at Raquette River (1.50 µg/g) and the lowest levels were found at Cornwall Island (0.88 µg/g) (Table 4.3). There were no correlations between renal retinol and contaminant concentrations or TEQs.

There were no significant differences among sites for renal retinyl palmitate (log transformed) ($p=0.12$, $H=11.5$) (Table 4.3). However, the highest mean concentrations were found in chicks from Raquette River (5.93 µg/g $\pm$ 6.14), and the lowest concentrations were found in chicks from Grasse River N (1.05 µg/g $\pm$ 0.98) (Table 4.3).

There were no correlations between renal retinyl palmitate and contaminant concentrations or TEQs.

There were no significant differences among sites for retinol:retinyl palmitate ratio ($p=0.62$, $H=5.3$) (Table 4.4). The highest ratio was found in chicks from Grasse River S (3.4) compared to low levels in chicks from Raquette River (0.80) (Table 4.4). There were no correlations between renal ratio and contaminant concentrations or TEQs.

Liver and kidney

Vitamin A parameters were correlated. Renal retinyl palmitate and renal retinol were positively correlated ($p=0.0005$, $r=0.79$) (Figure 4.4a). Hepatic retinol and molar hepatic ratio of retinol:retinyl palmitate were positively correlated ($p=0.00008$, $r=0.84$) (Figure 4.4b). With the addition of a bonferonni adjustment ($p=0.007$) there is still a postive correlation between hepatic retinol and molar hepatic ratio of retinol:retinyl palmitate.

4.3.3. Temporal trends for Vitamin A

There were no significant differences among years for hepatic retinol within most sites. However, hepatic retinyl palmitate were significantly different among years for some of the sites (Cornwall Island ($p=0.003$, $F=12$), Turtle Creek ($p=0.02$, $F=7.4$), and Cooper Marsh ($p=0.003$, $F=12$)). Chicks from Cornwall Island had liver retinyl palmitate levels higher in 1999 (83.36 μg/g) compared to 2000 (41.71 μg/g). Cooper Marsh birds contained 146.84 μg/g in 1999 compared to 62.90 μg/g in 2000. Turtle Creek birds were also higher in 1999 (120.76 μg/g) than 2000 (68.90 μg/g).

Renal retinol was not significantly different among sites between 1999 and 2000. Similarly, renal retinyl palmitate were not variable among sites, except Cooper Marsh.

Retinol and β – carotene in insects

Levels of retinol were highest at Turtle Creek (0.24 μg/g) compared to Cooper Marsh (0.08 μg/g) and Cornwall Sewage Treatment Plant (0.06 μg/g) (Figure 4.5). Levels of β–carotene were highest in chicks from Grasse River N and Cornwall Sewage Treatment plant (8.0 μg/g) compared to low levels at Hoasic Creek (2.0 μg/g) (Figure 4.6). There were no significant correlations between hepatic and renal mean retinol and mean retinyl palmitate with insect retinol and insect β–carotene.

Table 4.1. Mean (± standard deviation) hepatic retinol, and retinyl palmitate, renal retinol and retinyl palmitate (μg/g) in 16 day old tree swallow nestlings collected from the St. Lawrence River.

1999

SITE (*)	Liver Retinol Mean	Liver Retinol SD	Liver Retinyl palmitate Mean	Liver Retinyl palmitate SD	Kidney retinol Mean	Kidney retinol SD	Kidney Retinyl palmitate Mean	Kidney Retinyl palmitate SD
Grasse River N (c)	3.40	2.12	54.91 a	30	0.35 c	0.07	1.15 b	1.20
Cornwall Island (c)	6.10	3.56	83.36 ab	29	0.61 bc	0.15	2.81b	1.97
Hoasic Creek (r)	5.83	1.85	121.78 b	26	0.70 ab	0.14	2.30 b	0.84
Cooper Marsh (r)	6.22	3.32	146.84 bc	69	1.04 a	0.29	5.21 a	2.91
Turtle Creek (c)	7.23	3.08	120.76 b	28	0.76 b	0.18	5.58 a	5.42
Grasse River S (c)	5.76	1.88	65.97 a	22	0.60 b	0.10	1.56 b	0.70
Sewage Treatment (c)	5.02	2.81	74.25 ab	52	0.61 b	0.24	2.68 b	1.57

* c – contaminated/AOC site
* r - reference site
Significant differences among sites denoted by a,b,c ($p<0.05$).

Table 4.2. Mean molar ratio (± Standard deviation) of renal and hepatic retinol:retinyl palmitate (R:RP) in 16 day old tree swallows.

1999

	Ratio R:RP K		Ratio R:RP L	
Site ()	Mean	SD	Mean	SD
UC Bird Sanctuary (c)	0.60	0.30	0.10 bc	0.03
Grasse River North (c)	1.10	1.04	0.11 bc	0.01
Cornwall Island (c)	0.51	0.20	0.14 ab	0.07
Hoasic Creek (r)	0.58	0.10	0.09 bc	0.02
Cooper Marsh (r)	0.42	0.16	0.08 c	0.03
Turtle Creek (c)	0.49	0.38	0.11 bc	0.04
Grasse River South (c)	0.77	0.23	0.19 a	0.02
Sewage Treatment P(c)	0.49	0.18	0.12 bc	0.03

(c) - contaminated site/AOC site
(r) - reference site
UC - Upper Canada
Significant differences among sites denoted by a,b,c ($p<0.05$).

Table 4.3. Mean (± standard deviation) hepatic retinol, and retinyl palmitate, renal retinol and retinyl palmitate (μg/g) in 16 day old tree swallow nestlings collected from the St. Lawrence River.

2000

	Liver Retinol		Liver Retinyl Palmitate		Kidney retinol		Kidney Retinyl Palmitate	
SITE (*)	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Grasse River N (c)	7.45	5.57	67.27 ab	29.60	1.27	0.35	1.05	0.98
Cornwall Island (c)	4.60	4.42	41.71 b	12.93	0.88	0.50	2.12	1.28
Hoasic Creek (r)	9.46	3.34	87.91 ab	29.75	1.40	0.48	3.00	2.06
Cooper Marsh (r)	5.15	2.16	62.90 b	29.73	1.02	0.41	1.91	1.39
Turtle Creek (c)	7.93	7.15	68.90 b	40.97	1.15	0.89	2.39	2.21
Grasse River S (c)	13.50	3.91	93.33 ab	32.92	1.40	0.00	2.23	1.77
Sewage Treatment (c)	8.46	6.60	92.10 ab	42.84	1.15	0.70	3.17	2.68
Raquette River (c)	9.71	4.62	118.10 a	40.75	1.50	0.92	5.93	6.14

* c - contaminated site/AOC site

* r - reference site

significant differences among sites denoted by a, b ($p<0.05$)

Table 4.4. Mean molar ratio (± Standard deviation) of renal and hepatic retinol:retinyl palmitate (R:RP) in 16 day old tree swallows.

2000

	Ratio R:RP K		Ratio R:RP L	
	Mean	SD	Mean	SD
Grasse River North (c)	3.3 a	1.5	0.19	0.1
Cornwall Island (c)	1.1 b	0.5	0.19	0.1
Hoasic Creek (r)	1.1 b	0.5	0.21	0.1
Cooper Marsh (r)	1.2 b	0.5	0.16	0.0
Turtle Creek (c)	1.2 b	0.9	0.20	0.1
Grasse River South (c)	3.4 a	1.4	0.30	0.2
Sewage Treatment (c)	0.9 b	0.5	0.16	0.1
Raquette River (c)	0.8 b	0.6	0.17	0.1

(c) - contaminated site
(r) - reference site
Significant differences among sites denoted by a, b ($p<0.05$).

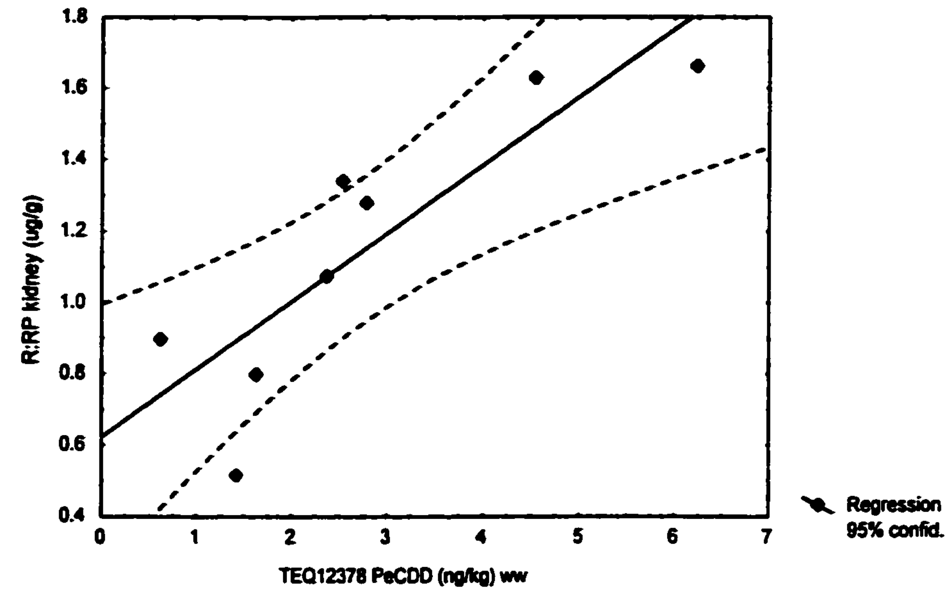
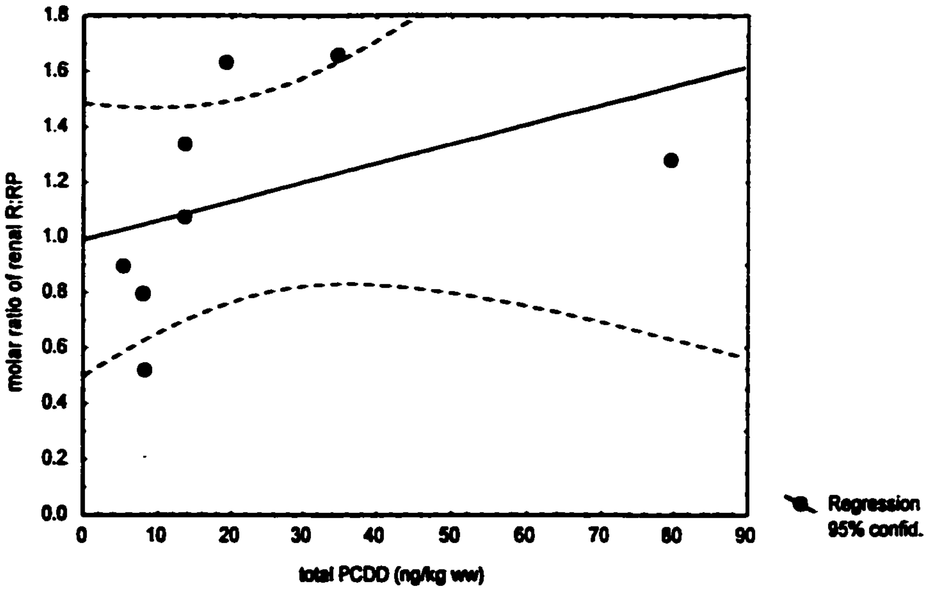
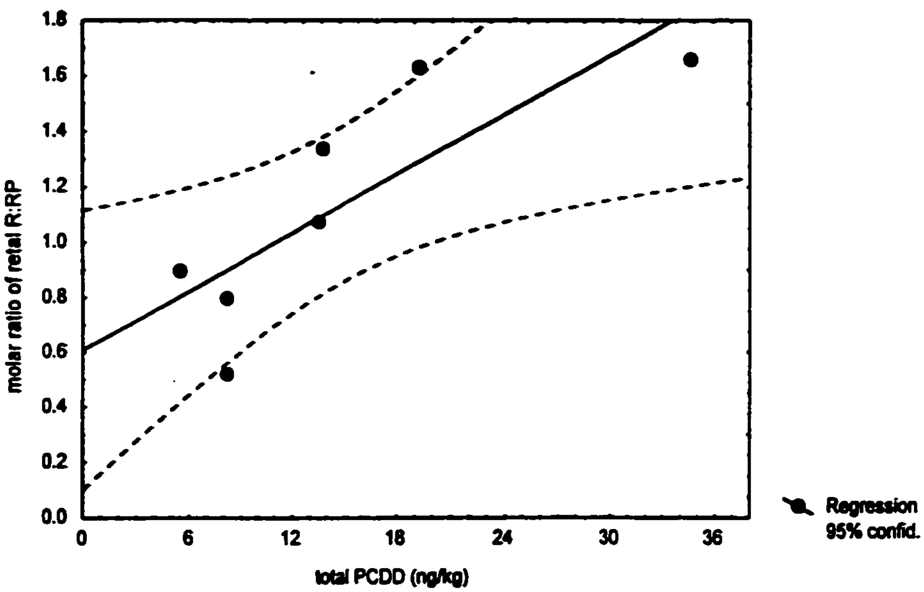


Figure 4.1. Spearman Rank between molar ratio of retinol:retinyl palmitate and TEQ 12378 PeCDD (ng/kg ww) in kidneys of 16 day old tree swallows (1999) ($p=0.002$, $r=0.90$). Based on 1 pool of 3 chicks/site.



[A]



[B]

Figure 4.2. [A] molar ratio of renal retinol:retinyl palmitate and total polychlorinated dibenzodioxin (ng/kg ww) in 16 day old tree swallows from the St. Lawrence River (1999), p=0.0285, r=0.761; and [B] spearman rank correlation between molar ratio of renal retinol:retinyl palmitate and total polychlorinated dibenzodioxin (ng/kg ww) in 16 day old tree swallows from the St. Lawrence River without 79 ng/kg ww TCDD outlier (1999), p=0.013, r=0.857. Based on 1 pool of 3 chicks/site.

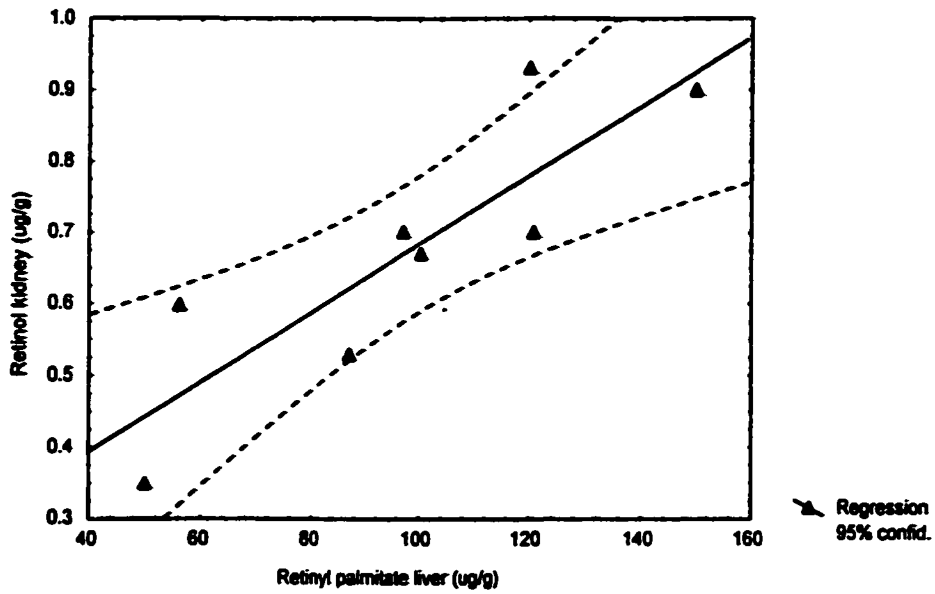
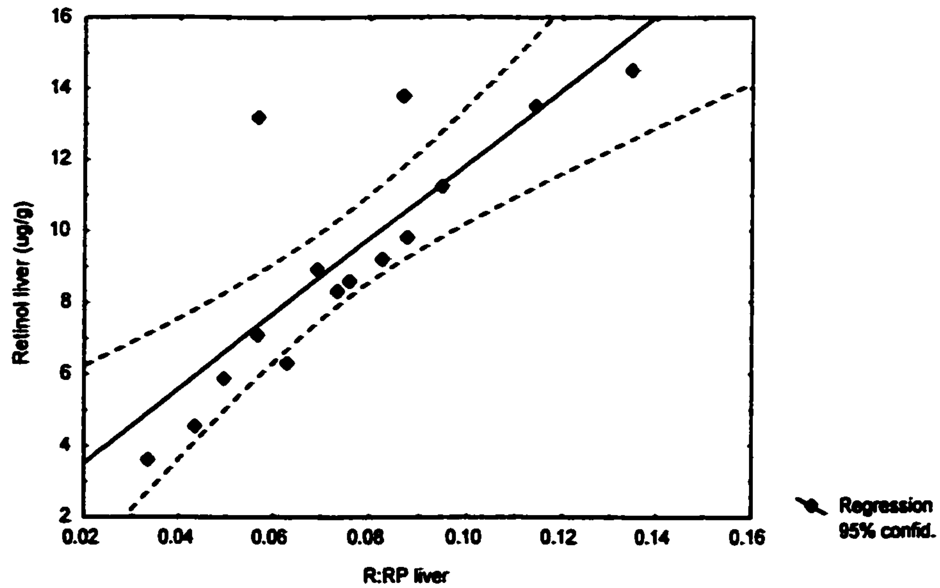
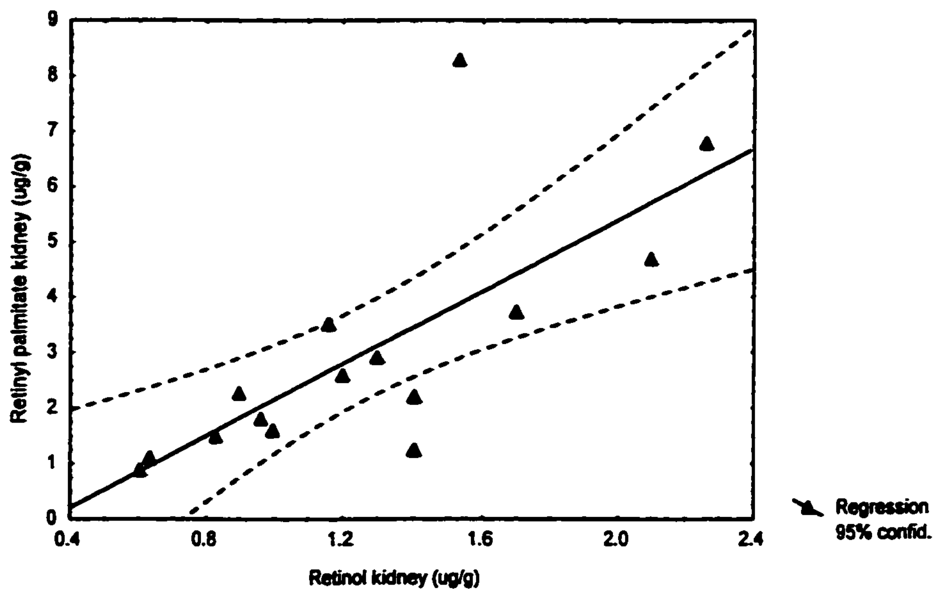


Figure 4.3. Spearman rank correlation between mean liver retinol palmitate and mean kidney retinol (µg/g) in 16 day old tree swallows collected from the St. Lawrence River (1999), $p=0.007$, $r=0.85$.



[A]



[B]

Figure 4.4. Spearman rank correlation between [A] hepatic retinol and hepatic molar ratio of retinol:retinyl palmitate, $p=0.00008$, $r=0.84$, and [B] renal retinol (μg/g) and renal retinyl palmitate (μg/g) in 16 day old tree swallows collected from the St. Lawrence River, $p=0.0005$, $r=0.79$ (2000).

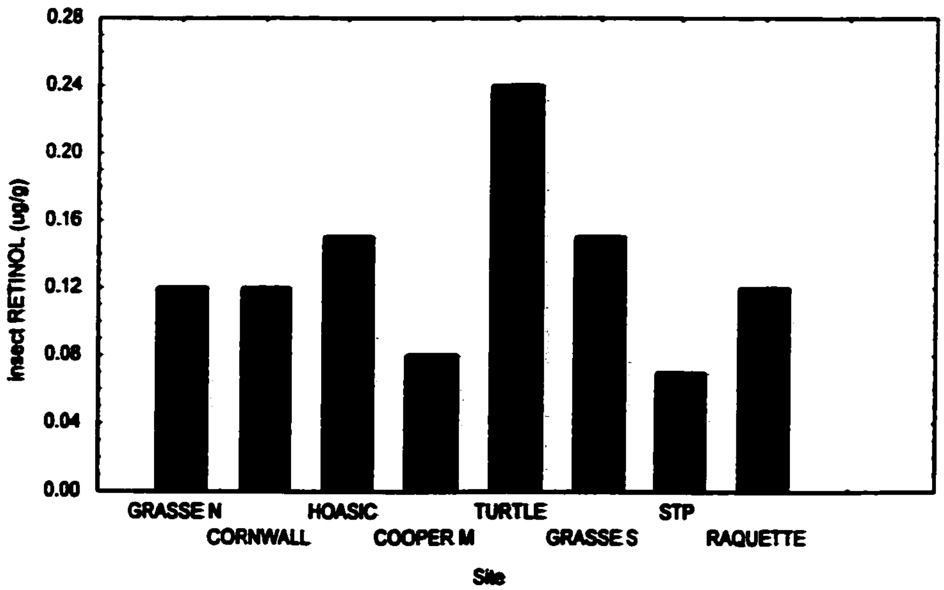


Figure 4.5. Pooled retinol (µg/g) collected from 8 day old tree swallow boluses from St. Lawrence River (2000). Based on 1 pool of 3 chicks per site.

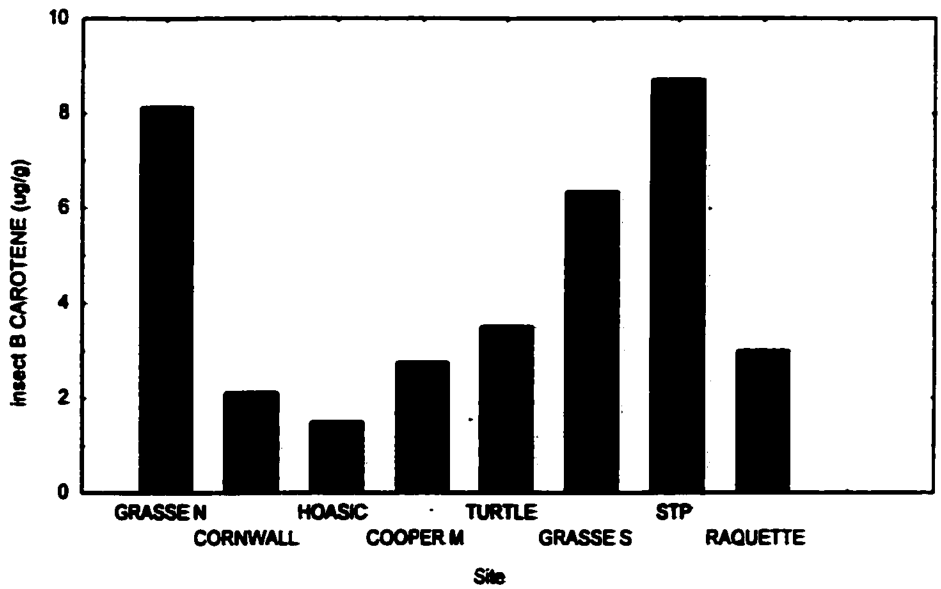


Figure 4.6. Pooled β-carotene (μg/g) collected from 8 day old tree swallow boluses from St. Lawrence River (2000). Based on 1 pool of 3 chicks per site.

4.4 Discussion

Our results demonstrate that the molar ratio of retinol:retinyl palmitate in the kidney was positively correlated with total dioxin. This is the first study on wildlife exposed to environmental contaminants to find a correlation between dioxin and renal levels of vitamin A in nestling tree swallows.

The majority of past studies that have found positive correlations between PCDD and the molar ratio of retinol:retinyl palmitate have been in egg yolk and plasma. Spear et al. (1990) collected herring gull eggs during early incubation from seven colonies in Lakes Huron, Ontario and Erie and measured for retinoid content and 2,3,7,8 TCDD yolk burden (14 ng/kg ww to 137 ng/kg ww). They found that the molar ratio of retinol:retinyl palmitate was positively correlated with 2,3,7,8-TCDD, and with the sum of the PCDD and PCDF toxic equivalents. Eight common tern colonies were examined for a correlation between exposure to PCDDs and PCDFs, and alterations in plasma retinoids, plasma thyroid hormone levels, and breeding parameters (Murk et al. 1996). They found a decrease in thyroid hormones, plasma retinoids and an increase in the ratio of plasma retinol in hatchlings to yolk sac retinyl palmitate. However, dietary vitamin A was not accounted for in this study, making circulating and storage forms of vitamin A difficult to interpret. In addition, retinyl palmitate changes through the course of incubation (Spear et al. 1990), which may change interpretation of results.

Currently, a higher ratio of retinol:retinyl palmitate seen in both laboratory and field avian studies suggests that there is an enhanced mobilization of vitamin A from the liver. This may explain the positive relationship seen between hepatic retinyl palmitate and renal retinol in tree swallows nesting along the St. Lawrence River in both years of the study. Higher levels of retinyl palmitate may lead to increased production of retinol which is then circulated through the body in the plasma. This elevated retinol level is then subjected to increased filtration through the kidneys, leading to elevated renal vitamin A. Elevated levels of retinol may also indicate an interference in the conversion of retinol to retinoic acid, or esterification in the liver, leading to elevated vitamin A circulating in the plasma (Green and Green 1994).

Much research has been devoted to the study of environmental contaminants and its impact on vitamin A levels. Although most of this research establishes a correlation between the depletion of vitamin A and exposure to contaminants, few studies have found an effect of PCBs and/or dioxins on renal vitamin A. Over 95% of vitamin A is stored as retinyl palmitate in the liver. Upon requirement for bone modulation and growth and other processes, this ester form of vitamin A is converted to retinol in the liver. However, only recently have there been findings to suggest a greater role of vitamin A circulation in the kidney. The kidney is a site of retinol binding protein (RBP) synthesis, and the renal RBP may play an important role in recycling retinol that is resorbed by the kidney (Green and Green 1994).

Chu et al. (2001) found that 1000 ng TCDD/kg bw in rats caused increased vitamin A levels in the kidney, and depleted levels of vitamin A in the liver. A similar trend has been noted in other laboratory studies (Chen et al. 1992). Although this may partially explain the positive correlation between renal retinol:retinyl palmitate and TCDD in this study, one would also expect to see a negative correlation between renal retinol:retinyl palmitate and hepatic retinol. Because the liver is the primary organ involved in synthesis of circulating vitamin A, there are larger quantities found in this organ compared to the kidney. This may offer an explanation of the sensitivity of the kidney to changes in vitamin A.

The current study has shown a positive correlation between renal retinol and hepatic retinyl palmitate. The liver is involved in the interconversion of retinyl palmitate to retinol which binds to retinol binding protein and transthyretin for plasma circulation (Green and Green 1994). Once unbound from the carrier protein complex, retinol enters the kidney for oxidation into inactive products which are released into the urine. Conceivably, high levels of retinyl palmitate may indicate high circulating levels of bound retinol in the plasma which would end up in the kidney. Alternatively, since some avian studies have found depleted levels of hepatic vitamin A, there may be increased filtration of retinol because of a displacement of retinol from the retinol binding protein.

Alternatively, enzymes involved in the synthesis and catabolism of vitamin A may also be affected by exposure to TCDD. An investigation by Nilson et al. (2000) found that dietary administration of 0, 0.1, 1, 10 or 100 μg/kg TCDD bw to rats resulted in increased renal lecithin:retinol acyltransferase (LRAT), increased renal retinol, decreased retinyl palmitate, and increased serum and renal levels of retinoic acid. It was suggested by these authors that transcription of retinoic acid-responsive genes were altered by dioxin. Cellular retinol binding protein (CRBP) may control cellular uptake of retinol from serum and provide LRAT with substrate, thereby regulating tissue retinyl palmitate levels (Levin and Davis 1997). Increased levels of retinol binding protein (RBP) in TCDD treated kidney would increase uptake of retinol from serum, and decrease renal retinyl palmitate via its effects on the LRAT enzyme. Additionally, hepatic retinol was not affected, and retinyl palmitate was reduced, but only at the 100 µg/kg TCDD, which was 10 fold higher than the dose required to affect renal vitamin A. Because the kidney also plays a major role in the filtration of retinol metabolic products (Hofmann and Eichlele 1994), it may be possible that an accumulation of retinol is the result of decreased enzyme activity involved in metabolic breakdown of retinol for filtration through the kidney.

In the current study, levels of vitamin A were significantly different between 1999 and 2000. Although dioxin levels were higher in 1999 (79 ng/kg ww compared to 2000 (34 ng/kg ww) (see Chapter 2), there was still a positive correlation between total PCDD and renal molar ratio of retinol:retinyl palmitate when levels of 34 ng/kg ww and lower were included in the 1999 statistical analysis. With the inclusion of 79 ng/kg TCDD, there appears to be a slight decrease in vitamin A levels. It could be interpreted that moderate levels of dioxin cause an increase in vitamin A and that extremely high levels of dioxin may cause a decrease in vitamin A. Environmentally exposed animals are often subjected to a plethora of contaminants with the potential for agonistic and antagonistic interactions on mechanisms responsible for vitamin A circulation. For example, high doses (400 to 1000 µmol/kg) 2,2',4,4',5,5' HeCB partially inhibited TCDD induced EROD activity and immunotoxicity in laboratory mice (Safe 1994). However, it is unknown how interactions between various chemicals affect wild populations of birds, and more specifically vitamin A homeostasis. High inter-year variation has also been seen in the renal retinol content of tree swallow nestlings studied throughout the Great

Lakes by Bishop et al. (1999) with levels twice as high at Wye Marsh in 1993 (2.4 μg/g) compared to 1994 (1.0 μg/g).

Dietary deficiencies of vitamin A can depress retinol concentrations in birds and in the yolks of their eggs (Bermudez et al. 1993). Once dietary intake of β–carotene enters the lumen of the intestine it is hydrolyzed by digestive lipases and esterases. Bile and pancreatic secretions also aid in the release of vitamin as retinol (Hofman and Eichele 1994). Retinol and β–carotene were collected in food boluses from tree swallow nestlings in 2000. We found the highest levels of retinol in boluses from Turtle Creek (0.24 μg/g). In comparison of chicks to boluses, Turtle Creek hepatic retinoid levels for tree swallow nestlings were moderate. Interestingly, insect retinol was lowest at Cooper Marsh and Cornwall Sewage Treatment Plant. Tree swallow hepatic retinol were also lowest at Cooper Marsh, but moderately high in chicks from the sewage treatment plant site. β–carotene in insect boluses was highest at Grasse River North (8.11 μg/g), where hepatic levels of retinol in tree swallows were only moderate and comparable to Turtle Creek levels. Cooper Marsh, Cornwall Island and Hoasic Creek boluses contained the lowest levels of β–carotene. Consequently, dietary impact of retinol and β–carotene may account for low levels of hepatic retinol observed in chicks from Cooper Marsh. However, high levels of β–carotene in boluses from Grasse River N is not reflected in hepatic retinol levels of tree swallows from this site. In addition, there were no correlations between retinol and retinyl palmitate in tree swallow kidney and livers and retinol and β–carotene in the insect samples.

Past research has found that organochlorine contaminants have a role in both the depletion and increase of vitamin A in the plasma and liver of avian species (Rolland 2000). Our results are inconsistent with some of the research which has found decreased levels of vitamin A in birds environmentally exposed to organochlorine contaminants from the Great Lakes and St. Lawrence River (Spear et al. 1986; Bishop et al. 1999).

Murvoll et al. (1999) found a borderline ($p<0.05$-0.10) positive correlation between total PCBs lipid weight (17.99 ng/g) in yolk and plasma retinol of 1 day old shag chicks. Elliott et al. (2001) found a positive relationship between liver retinol and yolk PCBs in Osprey collected from British Columbia and Washington and Oregon, USA. The two studies provide some evidence for increased vitamin A in birds exposed to organochlorine contaminants which is consistent with the results of the current study.

4.5 Conclusion and Summary

We found the molar ratio of retinol:retinyl palmitate in tree swallow nestling kidneys was positively correlated to total dioxin in 16 day old tree swallow nestlings in 1999. Vitamin A in insect boluses were not correlated with tree swallow vitamin A, indicating a lack of effect from dietary sources of retinol and β-carotene. Altered vitamin A metabolism may lead to numerous effects on avian reproduction, growth, and immune function. Poultry fed vitamin A deficient diets developed poor body condition, decreased egg production, and hatchability of fertile eggs (review by Fox 1993). Because of vitamin A's role in defending mucous membranes from microbial colonization, and adsorption of antigens, vitamin A has also been termed the "anti-infection vitamin" (Davis and Sell 1989). The lack of consistent trends in vitamin A in the current study would suggest the need for more research examining effects of different organochlorine contaminants and mixtures on avian species.

Chapter 5

5.0 General Conclusions and Recommendations

This work illustrates that exposure to environmental organochlorine contaminants can disrupt the endocrine pathway leading to elevated levels of stress corticosterone, and alter kidney vitamin A levels in 16 day old tree swallows (*Tachycineta bicolor*) nesting along the shores of the St. Lawrence River.

Polychlorinated biphenyls (PCBs) were historically used by industries such as Reynolds Metals, Aluminum Company of America (ALCOA) and General Motors which are located on the St. Lawrence River and its tributaries. Present day landfill sites, storage lagoons and wastewater outfalls have all contributed to organochlorine contamination of the St. Lawrence, Grasse and Raquette River sediment. The three most contaminated sites studied were Turtle Creek, located directly adjacent to a General Motors PCB landfill site; and Grasse River North and South, located within the vicinity of ALCOA. Levels of total PCBs, non-ortho PCBs, polychlorinated dibenzodioxin (PCDD) and polychlorinated dibenzofuran (PCDF) and total TEQs were highest in tree swallow chicks collected from these three sites. However, many of the contaminants such as furans, total PCBs and non-ortho PCBs were higher in 2000 compared to 1999. There has been no dredging in the Grasse, Raquette or St. Lawrence Rivers since 1995 when ALCOA removed 2,600 cubic yards of sediment near a property outfall. Therefore no known manipulations of the sediment would account for changes in contaminant concentrations. In light of these results it is recommended that more chicks are analysed on an individual basis for dioxins, furans and non-ortho PCBs to provide a more representative sample of the site. Additionally, contaminant studies should be conducted over a number of years to establish trends. Factors which affect changes in contaminant dynamics such as sediment composition, weather, and prey community of the test organism should be considered in these studies.

Our results also found that the highest levels of total PCBs in sediment and insects were found at Turtle Creek and Grasse River, which is similar to the pattern for contaminant body burden in tree swallow nestlings. However, there were spatial variations and differences in BAFs (bioaccumulation factors) calculated from insect, sediment and tree swallow nestlings. In the present study areial invertebrates were collected at dusk for contaminant analysis. While sampling at night provides a quick sample of a large density of insects, tree swallows are primarily diurnal feeders. For future work it is recommended that sweep samples of diurnal insects and/or insect bolluses are collected from chicks at numerous times throughout the growth period for contaminant levels. This will be more labour intensive but will provide a more accurate account of contaminants from dietary sources.

Examination of basal and stress corticosterone found a positive correlation between ten minute stress corticosterone levels and total dioxins in 1999 and 2000. Since corticosterone is a key hormone in the initiation of gluconeogenesis and regulation of glucose homeostasis, it is probable that elevated corticosterone levels in animals exposed to TCDD are a response to "metabolic stress" related to glucose metabolism. It has been further proposed that this interaction causes corticosterone production to increase in response to low glucose (Gorski et al. 1988a). It is recommended that future work examine the mechanism of corticosterone and organochlorine exposure and incorporates analysis of plasma glucose and enzymes involved in gluconeogenesis such as PEPCK (phosphoenolpyruvate carboxykinase) to examine a wider scope of endocrine effects in tree swallows exposed to organochlorine contaminants.

Previous studies with tree swallow chicks along the St. Lawrence River had found a negative correlation between liver retinol and ethoxyresorufin-o-deethylase (EROD) activity, indicating contaminant exposure (Bishop et al. 1999). In the current study it was determined that the molar ratio of retinol to retinyl palmitate in the kidney was positively correlated with dioxin concentrations in 1999. Although the liver has been more impacted by contaminants in avian field research (Elliot et al. 2001; Bishop et al. 1999; Government of Canada 1991), the kidney has been proven to be sensitive to dioxins in rat studies. TCDD administered to to rats resulted in increased renal lecithin:retinol acyltransferase (LRAT), increased renal retinol, decreased retinyl palmitate, and increased serum and renal levels of retinoic acid. In future studies, other forms of vitamin A such as retinoic acid and retinal should also be analysed. If there is a disruption in the conversion of retinol to retinoic acid and retinal, there may be depleted or elevated levels of retinol in the liver and kidney. Additionally, enzymes such as LRAT should be examined to determine possible effects of contaminants on enzyme pathways.

It is apparent from the current study that tree swallows exposed to high levels of contaminants are still capable of high hatching and fledging success. However there may still be biochemical alterations in the chicks which lead to compromised health. Elevated levels of corticosterone may impact protein, lipid and carbohydrate metabolism, and have immunosuppressive effects. High glucocorticoids reduce the number of antibody producing cells and circulating lymphocytes (Ribelin 1984), which may lead to an increased incidence of infections by bacteria, protozoan in animals with hyper activity of corticosterone or cortisol. While body weights were unaffected in this study, another implication of elevated levels of corticosterone is its possible negative effects on growth and weight gain in birds. This steroid induces breakdown of both protein and nucleic acid and decreases protein synthesis to increase the amino acid substrate for gluconeogenesis. Long term exposure to PCDD may lead to increase wasting, characterized by weight loss in young chicks.

Altered vitamin A metabolism may lead to numerous effects on avian reproduction, growth, and immune function. Poultry fed vitamin A deficient diets developed poor body condition, decreased egg production, and hatchability of fertile eggs (review by Fox 1993). Because of vitamin A's role in defending mucous membranes from microbial colonization, and adsorption of antigens, vitamin A has also been termed the "anti-infection vitamin" (Davis and Sell 1989). Since tree swallows have greater than 60% nest fidelity, banding nestlings and revisiting nest sites in following years is recommended to assess potential success of first year survival and reproductive success and health of individual birds.

6.0 References

Ahlborg UG, Brouwer A, Fingerhut MA, Jacobson JL, Jacobson SW, Kennedy SW, Kettrup AAF, Koemon JH, Poiger H, Rappe C, Safe SH, Seegal RF, Tuomisto J, van den Berg M. 1992. Impact of polychlorinated dibenzo-p-dioxins, dibenzofurans, and biphenyls on human health, with special emphasis on application of the toxic equivalency factor concept. European. J. Pharmacol. Environ. Toxicol. Pharmacol. Sect. 228: 179-199.

Bentzen E, Mackay D, Hickie BE, Lean DRS. 1999. Temporal trends in polychlorinated biphenyls (PCBs) in Lake Ontario fish and invertebrates. Env. Rev. 7: 203-223.

Bermudez AJ, Swayne DE, Squires MA, Radin MJ. 1993. Effects of vitamin A deficiency on the reproductive system of mature white leghorn hens. Avian. Dis. 37: 274-283.

Bestervelt LL, Pitt JA, Nolan CJ, Piper WN. 1993. TCDD alters pituitary-adrenal function II: evidence for decreased bioactivity of ACTH. Neurotox. Teratology. 15: 371-376.

Bishop CA, Koster MD, Chek AA, Hussell DJT, Jock K. 1995. Chlorinated hydrocarbons an mercury in sediments, red-winged blackbirds (*Agelaius phoeniceus*) and tree swallows (*Tachycineta bicolor*) from wetlands in the Great Lakes-St. Lawrence river basin. Environ. Toxicol. Chem. 14: 491-501.

Bishop CA, Mahony NA, Trudeau S, Pettit KE. 1999. Reproductive success and biochemical effects in tree swallows (Tachycineta bicolor) exposed to chlorinated hydrocarbon contaminants in wetlands of the Great lakes and St. Lawrence river basin , USA and Canada. Environ. Toxicol. Chem. 18:263-271.

Bishop CA, Martinovic B. 2000. Guidelines and procedures for toxicological field investigations using amphibians and reptiles. In: Ecotoxicology of amphibians and reptiles, eds Sparling DW, Linder G, Bishop CA. Society of Environmental Toxicology and Chemistry (SETAC).

Blomhoff R. 1994. Overview of vitamin A metabolism and function. In: Vitamin A in health and disease. Ed Blomhoff R, Dekker M, New York pp 1-35.

Boily MH, Champoux DH, Bourbannais DH, DesGranges JL, Rodrigue J, Spear PA. 1994. B-carotene and retinoids in eggs of great blue herons (*Ardea herodias*) in relation to St. Lawrence river contamination. Ecotoxicology. 3: 271-286.

Breuner CW, Wingfield JC, Romero LM. 1999. Diel rhythms of basal and stress induced corticosterone in a wild, seasonal vertebrate, Gambel's white crowned sparrow. J. Exp. Zool. 284: 334-342.

Brouwer A, van den Berg KJ. 1986a. Binding of a metabolite of 3,4,3',4'-tetrachlorobiphenyl to transthyretin reduces vitamin A transport by inhibiting the formation of the protein complex carrying both thyroxin. Toxicol. Appl. Pharmacol. 85: 301-312.

Brouwer A, Vand den Berg KJ, Blaner WS, Goodman De WS. 1986. Transthyretin (preablumin) binding of PCBs, a model for the mechanism of interference with vitamin A and thyroid hormone metabolism. Chemosphere. 15: 1699-1706.

Brouwer A. 1991. Role of biotransformation in PCB-induced alterations in vitamin A and thyroid metabolism in laboratory and wildlife species. Biochem. Soc. Trans. 19: 731-737.

Bruener CW, Wingfield JC, Romero ML. 1999. Diel rhythms of basal and stress-induced corticosterone in a wild, seasonal vertebrate, Gambels' white-crowned sparrow. J. Exp. Zool. 284: 334-342.

Byrne JJ, Carbone JP, Pepe MG. 1988. Suppression of serum adrenal cortex hormones by chronic low dose polychlorobiphenyl or polybromobiphenyl treatments. Arch Environ. Contam. Toxicol. 17: 47-53.

Cecil HC, Harris SJ, Bitman J, Fries GF. 1973. Polychlorinated biphenyl-induced decreases in liver vitamin A in Japanese quail. Bull. Environ. Contam. Toxicol. 9: 179-185.

Chen LC, Berberian I, Koch B, Mercier M, Azais-Braesco V, Glauert HP, Chow CK, Robert LW. 1992. Polychlorinated and polybromated biphenyl congeners and retinoid levels on structure-activity relationships. Toxicol. Appl. Pharmacol. 114: 47-55.

Chiarenzelli J, Bush B, Casey A, Barnard E, Smith B, O'Keefe P, Gilligan E, Johnson G. 2000. Defining the sources of airborne polychlorinated biphenyls: evidence for the influence of microbially dechlorinated congeners from river sediment? Can. J. Fish. Aquat. Sci. 57(Suppl 1): 86-94.

Cho YC, Kim J, Sokol RC, Rhee GY. 2000. Biotransformation of polychlorinated biphenyls in St. Lawrence river sediments: reductive dechlorination and dechlorinating microbial populations. Can. J. Fish. Aquat. Sci. 57: 95-100.

Chu I, Poon P, Yagminas A, Lecavalier P, Hakansson H, Valli VE, Kennedy SW, Bergman, Seegan RF, Feeley M. 1998. Subchronic toxicity of PCB 105 (2,3,3',4,4' pentachlorobiphenyl) in rats. J. Appl. Toxicol. 18: 285-292.

Colby HD, Longhurst PA. 1996. Toxicology of the adrenal gland. In:Endocrine Toxicology (Atterwill CK, Flack JD, eds). Cambridge: Cambridge University Press. Pp 243-281.

Cuomo MM, Jorling TC. 1990. St. Lawrence river at Massena remedial action plan. Stage 1. New York State Department of Environmental conservation.

DeVoogt P, Wells De, Reutergardh L, Brinkman UATH. 1990. Biological activity, determination and occurrence of planar mono-, di-ortho PCBs. Intern. J. Environ. Anal. Chem. 40: 1-46.

DeWeese LR, Cohen RR, Stafford CJ. 1985. Organochlorine residues and eggshell measurements for tree swallows, *Tachycineta bicolor*, in Colorado. Bull. Environ. Contam. Toxicol. 35: 767-775.

DiBartolomeis MJ, Moore RW, Peterson RE, Christian BJ, Jefcoate CR. 1987. Altered regulation of adrenal steroidogenesis in 2,3,7,8-tetrachlorodibenzo-p-diosin-treated rats. Biochem. Pharm. 36: 59-67.

Drier SI, Anderson J, Biberhofer J, Eckersley M, Helliar R, Hickey MBC, Richman L, Stride F. 1997. Remedial action plan for the Cornwall-lake St. Francis area. Stage 2 report: The recommended plan. St. Lawrence Rap team.. ISBN: 0-7778-68881-4. Pp 203.

Elliott JE, Martin PA, Arnold TW, Sinclair PH. 1994. Organochlorines and reproductive success of birds in orchard and non-orchard areas of central British Columbia, Canada, 1990-1991. Arch. Environ. Contam. Toxicol. 26: 435-443.

Elliott JE, Wilson LK, Henny CJ, Trudeau SF, Leighton FA, Kennedy SW, Cheng KM. 2001. Assessment of biological effects of chlorinated hydrocarbons in osprey chicks. Environ. Toxicol. Chem. 20: 866-879.

Environment Canada. 1998. Canadian tissue residue guidelines for polychlorinated biphenyls for the protection of wildlife consumers of aquatic biota. Science policy and environmental quality branch. 66pp.

Fairchild WL, Muir DCG, Currie RS, Yarachewski AL. 1992. Emerging insects as a biotic pathway for movement of 2,3,7,8-tetrachlorodibenzo-p-dioxin from lake sediments. Environ. Toxicol. Chem. 11:867-872.

Fox GA. 1993. What have biomarkers told us about the effects of contaminants on the health of fish-eating birds in the Great Lakes? The theory and a literature review. J Great. Lakes. Res. 19: 722-736.

Froese KL, Verbrugge DA, Ankley GT, Niemi GJ, Larsen CP, Giesy JP. 1998. Bioaccumulation of polychlorinated biphenyls from sediment to aquatic insects and tree swallow eggs and nestlings in Saginaw Bay, Michigan, USA. Environ. Toxicol. Chem. 17: 484-492.

Gendron AD, Bishop CA, Fortin R, Hontela A. 1997. In vivo testing of the functional integrity of the corticosterone-producing axis in mudpuppy (amphibia) exposed to chlorinated hydrocarbons in the wild. Env. Toxicol. Chem. 16: 1694-1706.

Gerrard PM, St. Louis VL. 2001. The effects of experimental reservoir creation on the bioaccumulation of methylmercury and reproductive success of tree swallows (*Tachycineta bicolor*). Environ. Sci. Technol. 35: 1329-1338.

Gilbertson M, Kubiak T, Lidwig J, Fox G. 1991. Great Lakes embryo mortality, edema, and deformities syndrome (GLEMEDS) in colonial fish-eating birds: Similarity to chick-edema disease. J. Toxicol. Environ. Health. 33: 455-520.

Gorski JR, Muzi G, Weber LW, Pereira DW, Iatropoulos MJ, Rozman K. 1988a. Elevated plasma corticosterone levels and histopathology of the adrenals and 2,3,7,8-tetrachlorodibenzo-p-dioxin treated rats. Toxicol. 53: 19-32.

Gorski JR, Rozman T, Griem H, Rozman K. 1988b. Corticosterone modulates acute toxicity of 2,3,7,8- tetrachlorodibenzo-p-dioxin in male sprague-dawley rats. Fundam. Appl. Toxicol. 11: 494-502.

Gorski JR, Weber LWD, Rozman K. 1990. Reduced gluconeogenesis in 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) treated rats. Arch .Toxicol. 64: 66-71.

Government of Canada. 1991. Toxic chemicals in the Great Lakes and associated effects, Vol II - Effects Supply and Services Canada. Canadian Wildlife Service, p58.

Green MH, Green JB. 1994. Dynamics and control of plasma retinol. In Blomhoff R, ed, Vitamin A in health and disease. Marcel Dekker, New York, NY, USA. Pp. 119-133.

Hansen L. 1987. Environmental toxicology of polychlorinated biphenyls, in Polychlorinated biphenyls (PCBs):Mammalian and environmental toxicology, environmental toxin series, Safe S, Hutzinger O, Eds, Springer-Verlag, Heidelberg, Pp. 15.

Harris MP, Osborn D. 1981. Effect of a polychlorinated biphenyl on the survival and breeding of puffins. J. Appl. Ecol. 18: 471-479.
Health. Persp. 107: 179-186.

Hebert CE, Bishop CA, Weseloh DV. 1993. Evaluation of wetland biomonitors for the Great Lakes: a review of contaminant levels and effects in five vertebrate classes. Canadian Wildlife Service Technical Report Series No. 182. 103 pp.

Hofmann C, Eichlele G. 1994. Retinoids in development. In Sporn MB, Roerts AB, Goodman DS, eds, The retinoids: Biology, chemistry, and medicine. Raven, New York, NY, USA. Pp 239-245.

Holberton RL, Parrish JD, Wingfield JC. 1996. Modulation of the adrenocortical stress response in neotropical migrants during autumn migration. The Auk. 113: 558-564.

Hontela A, Dumont P, Duclos D, Fortin R. 1995. Endocrine and metabolic dysfunction in yellow perch, *perca flavescens*, exposed to organic contaminants and heavy metals in the St. Lawrence River. Environ. Toxicol. Chem. 14: 725-731.

Hontela A, Rasmussen JB, Audet C, Chevalier G. 1992. Impaired cortisol stress response in fish from environments polluted by PAH, PCBs, and mercury. Arch Environ. Contam. Toxicol. 22: 278-283.

Hontela A. 1997. Endocrine and physiological responses of fish to xenobiotics:role of glucocorticoid hormones. Rev. Toxicol. 1: 1-46.

Hopkins WA, Mendonca MT, Congdon JD. 1999. Responsiveness of the hypothalamo-pituitary-interrenal axis in an amphibian (*Bufo terrestris*) exposed to coal combustion wastes. Comp. Biochem. Physiol. C 122: 191-196.

Hussell DJT, Quinney TE. 1987. Food abundance and clutch size of tree swallows. Ibis. 129: 243-258.

Jones PD, Giesy, Newsted JL, Verbrugge DA, Beaver DL, Ankley GT, Tillitt DE, Lodge KB, Niemi GJ. 1993. 2,3,7,8-tetrachlorodibenzo-p-dioxin equivalents in tissues of birds at Green Bay, Wisconsin, USA. Arch. Environ. Contam. Toxicol. 24: 345-354.

Kennedy SW, Lorenzen A, Jones SP, Hahn ME, Stegeman JJ. 1996(a). Cytochrome P4501A induction in avian hepatocyte cultures: a promising approach for predicting the sensitivity of avian species to toxic effects of halogenated aromatic hydrocarbons. Toxicol. Appl. Pharmacol. 141: 214-230.

Kennedy SW, Lorenzen A, Norstrum RJ. 1996b. Chicken embryo hepatocye bioassay for measuring cytochrome P4501A-based 2,3,7,8-tetrachlorodibenzo-p-dioxin equivalent concentrations in environmental samples. Environ. Sci. Tech. 30: 706-715.

Keul DW, Butterworth BC, McBride A, Kroner S, Bahnick D. 1989. Contamination of fish by 2,3,7,8-tetrachlorodibenzo-p-dioxin: a survey of fish from major watersheds in the United states. Chemosphere. 18: 1997-2003.

Korach KS, Sarver P, Chae K, Mclachlan JA, McKinney JD. 1988. Estrogen receptor binding activity of polychlorinated hydroxybiphenyls:conformationally restricted structural probes. Mol. Pharmacol. 33: 120-127.

Kubiak TJ, Harris HJ, Smith LM, Schwartz TR, Stalling DL, Trick JA, Sileo L, Docherty DE, Erdman TC. 1989. Microcontaminants and reproductive impairment of the Forster's terns on Green Bay, Lake Michigan - 1983. Arch. Environ0 Contam0 Toxicol. 18: 706-727.

Kuratsune M. 1989. Yusho, in Halogenated biphenyls, terphenyls, naphthalenes, dibenzodioxins and related products ,Kimbrough RD, Ed., Elsevier/North Holland, Amsterdam, Pp. 287.

Lamparski LL, Nestrick TJ, Stenger VA. 1984. Presence of chlorodibenzo dioxins in a sealed 1933 sample of municipal sludge. Chemosphere. 13: 361-368.

Lean DRS. 2000. Some secrets of a great river: an overview of the St. Lawrence River supplement. Can. J. Fish. Aquat. Sci. 57:1-6.

Leboulenger F, Delarue C, Belanger A, Perroteau I, Netchitailo P, Leroux P, Jegou S, Tonon MC, Vaudry H. 1982. Direct radioimmunoassays for plasma corticosterone and aldosterone in frog i. Validation of the methods and evidence for daily rhythms in a a natural environment. Gen. Comp. Endocrin. 46: 521-532.

Levin MS, Davis AE. 1997. Retinoic acid increased cellular retinol binding protein II and mRNA and retinol uptake in the human intestinol caco-2 cell line. J. Nutr. 127: 13-17.

Lorenzen A, Moon TW, Kennedy SW, Fox GA. 1999. Relationships between environmental organochlorine contaminant residues, plasma corticosterone concentrations, and intermediary metabolic enzyme activities in great lakes herring gull embryos. Environ. Health. Persp. 107: 179-186.

Ludwig JP, Auman HJ, Kurita H, Ludwig ME, Campbell LM, Giesy JP, Tillit DE, Jones P, Yamashita N, Tanabe S, Tatsukawa R. 1993. Caspian tern reproduction in the Saginaw bay ecosystem following a 100 yr flood event. J. Great. Lakes. Res. 19: 96-108.

McArthur MLB, Fox GA, Peakall DB, Philogene BJR. 1983. Ecological significance of behavioural and hormonal abnormalities in breeding ring doves fed an organochlorine chemical mixture. Arch Environ Contam Toxicol, 12:343.

McCarty JP, Secord AL. 1999. Reproductive ecology of tree swallows (*Tachycineta bicolor*) with high levels of polychlorinated biphenyl contamination. Environ. Toxicol. Chem. 18:1433-1439.

McCarty JP, Secord AL. 1997. Polychlorinated biphenyl contamination of tree swallows in the Upper Hudson river valley, New York. New York Field Office, US Fish and Wildlife Service, Cortland, New York.

McNeil R. 1997. Whole body processing of birds. Procedures Manual, Laboratory Services Section, NWRC. SOP-TP-PROC-06B.

Meserve LA, Murray BA, Landis JA. 1992. Influence of maternal ingestion of Aroclor 1254 (PCB) or Firemaster BP-6 (PCB) on unstimulated and stimulated corticosterone levels in yound rats. Bull. Environ. Contam. Toxicol. 48:715-720.

Miller DB, Gray LE Jr, Andrews JE, Lucbke RW, Smialowiwcz RJ. 1993. Repeated exposure to the polychlorinated biphenyl (Aroclor 1254) elevates the basal serum levels of corticosterone but does not affect the stress induced-rise. Toxicology 81:217-222.

Mitchell MF, McLeod HA, Roberts JR. 1984. Polychlorinated dibenzofurans: Criteria for their effects on humans and the environment. National Research Council Canada. Associate Committee on Scientific Criteria for Environmental Quality. ISSN 0316-0114. Pp 243.

Murk AJ, Boudewijn TJ, Meininger PL, Bosveld ATC, Rossaert G, Ysabaert T, Meire P, Dirksen S. 1996. Effects of polyhalogenated aromatic hydrocarbons and related contaminants on common tern reproduction: integration of biological biochemical and chemical data. Arch. Environ. Contam. Toxicol. 31: 128-140.

Murvoll KM, Skaare JU, Nilssen VH, Bech C, Ostnes JE, Jenssen BM. 1999. Yolk PCB and plasma retinol concentrations in shag (*P. aristoten)*. Arch. Environ. Contam. Toxicol. 36: 308-315.

Naf C, Broman D, Ishaq R, Yebuhr Y. 1990. PCDDs and PCDFs in water, sludge and air samples from various levels in a waste water treatment plant with respect to composition changes and total flux. Chemosphere. 20: 1503-1510.

Nichols JW, Larsen CP, McDonald ME, Niemi GJ, Ankley GT. 1995. A bioenergetics-based model for accumulation of polychlorinated biphenyls by nesting tree swallows, *Tachycineta bicolor*. Environ. Sci. Technol. 29: 604-612.

Nilson CB, Hoeberg P, Trossvik C, Azais-Braesco V, Blaner WS, Fox G, Harrsion EH, Nau H, Schmidt CK, van Bennekum AM, Hakansson H. 2000. 2,3,7,8-tetrachlorodibenzo dioxin increases serum and kidney retinoic acid levels and kidney retinol esterification in the rat. Toxicol. Appl. Pharmacol. 1: 121-131.

Norris DO. 1997. Vertebrate endocrinology, 3rd edition. Academic Press. Pp. 329-353.

Nostrom RJ, Simon M. 1991. Determination of specific polychlorinated dibenzo-p-dioxin and dibenzofurans in biological matrices by gel-permeation/carbon chromatography and gas chromatography-mass spectrometry. In Rappe C, Buser HR, Dodet B, O'Neill IK (eds). Environmental carcinogens, methods of analysis and exposure measurement Vol. 11 Polychlorinated dibenzo-p-dioxins and dibenzofurans. World Health Organization, International Association for Research on Cancer, Lyon, France, Pp. 281-297.

NYSDEC. 1990. Draft St. Lawrence river at Massena remedial action plan- stage 1. New York State Department of Environmental Conservation, Albany, New York, May 1990.

Poland A, Knutson JC. 1982. 2,3,7,8-tetrachlorodibenzo-p-dioxin and related halogenated aromatic hydrocarbons: examination of the mechanism of toxicity. Ann Rev Pharmacol. Toxicol. 22:517-554.

Quinney TE, Ankney CD. 1985. Prey size selection by tree swallows. Auk. 102: 245-250.

Ribelin WE. 1984. The effects of drugs and chemicals upon the structure of the adrenal gland. Fundam. Appl. Toxicol. 4: 105-119.

Robertson RJ, Stutchbury BJ, Cohen RR. 1992. Tree Swallow. In the Birds of North America, no. 11 Poole A, Stettenheim P, Gill F, eds. Philadelphia: the Academy of Natural Sciences; Washington DC: The American Ornithologist' Union.

Rolland RM. 2000. A review of chemically-induced alterations in thyroid and vitamin A status of wildlife and fish. J. Wildl. Dis. 36: 615-635.

Safe SH. 1994. Polychlorinated biphenyls (PCBs): environmental impact, biochemical and toxic responses, and implications for risk assessment. Critical. Rev. Toxicol. 24:87-149.

Scheuhammer AM, Bond D. 1991. Factors affecting the determination of total mercury in biological samples by continuous-flow cold vapor atomic absorption spectrophotometry. Biol. Trace. Elem. Res. 31: 119-129.

Schwabl H, Wingfield JC, Farner DS. 1980. Seasonal variation in plasma levels of luteinizing hormone and steroid hormones in the european blackbird, *Turdus merula*. Die. Vogelwarte. 30: 283-294.

Secord AL, McCarty JP. 1997. Polychlorinated biphenyl contamination of tree swallows in the Upper hudson river valley, New York. New York Field Office. US Fish and Wildlife Service, Cortland, New York.

Shaw GC. 1983. Organochlorine pesticide and PCB residues in eggs and nestlings of tree swallows, *Tachycineta bicolor*, in Central Alberta. Can. Field-Naturalist 98: 258-260.

Simms W, Jeffries S, Ikonomou M, Ross PS. 2000. Contaminant-related disruption of vitamin A dynamics in free ranging harbour seal (*Phoca vitulina*) pups from British Columbia, Canada, and Washington State, USA. Environ. Toxicol. Chem. 19: 2844-2849.

Simon J. 1984. Effects of daily corticosterone injections upon plasma glucose, insulin, uric acid and electrolytes and food intake pattern in the chicken. Diabete Metabol. 10:211-217.

Sloan RJ, Jock K. 1990. Chemical contaminants in fish from the ST. Lawrence river drainage on lands of the Mohawk nation at Akwasasne and near the General motors corporation/central foundry division Massena, New York. Technical report 90-1 (BEP). Division of Fish and Wildlife. Pp 87.

Sokol RR, Rohf FJ. 1981. Biometry, 2nd ed. WH Freeman, New York, NY, USA.

Sokol RC, Kwon OS, Bethoney CM, Rhee GY. 1994. Reductive dechlorination of polychlorinated biphenyls (PCBs) in St. Lawrence river sediments and variations in dechlorination characteristics. Environ. Sci. Technol. 28: 2054-2064.

Spear PA, Bourbonnais DH, Norstrom RJ, Moon TW. 1990. Yolk retinoids (vitamin A) in eggs of the herring gull and correlations with polychlorinated dibenzo-p-dioxin and dibenzofurans. Environ. Toxicol. Chem. 9: 1053-1061.

Spear PA, Garcin H, Narbonne JF. 1988. Increased retinoic acid metabolism following 3,3',4,4',5,5'-hexabromobiphenyl injection. Can. J. Physiol. Pharmacol. 66: 1181-1186.

Spear PA, Moon TW, Peakall DB. 1986. Liver retinoid concentrations in natural populations of herring gulls (Larus argentatus) contaminated by 2,3,7,8-tetrachlorodibenzo-p-dioxin and in ring doves (Streptopelia risoria) injected with a dioxin analogue. Can. J. Zool. 64: 204-208.

Stahl BU, Beer DG, Weber LW, Rozman K. 1993. Reduction of hepatic phosphoenolpyruvate carboxykinase (PEPCK) activity by tetrachlorodibenzo-p-dioxin (TCDD) is due to decreased mRNA levels. Toxicol. 79: 81-95.

Statsoft Inc. 1997 STATISTICA for windows computer program manual. Tulsa, OK.

Stone WB, Jock K, Gradoni PB. 1991. Chemical contaminants in wildlife from the Mohawk nation at Akwasasne and the vicinity of the General motors corporation/central foundry division Massena, New York plant. Draft. New York State Department Environmental Conservation, New York. Pp. 151

Suns K, Hitchin G, Toner D. 1991. Spatial and Temporal trends of organochlorine contaminants in spottail shiners (Notropis Hudsonius) from the Great Lakes and their connecting channels (1975-1988). Water Resources Branch, Environment Ontario, Toronto, Ontario.

Tracey GA, Hansen DJ. 1996. Use of biota-sediment accumulation factors to assess similarity of nonionic organic chemical exposure to benthically-coupled organisms of differing trophic mode. Arch. Environ. Contam. Toxicol. 30: 467-475.

Turle RT, Norstrom RJ, Collins B. 1991. Comparison of PCB quantitation methods: re-analysis of archived specimens of herring gull eggs from the Great Lakes. Chemosphere 22: 201-213.

Underwood BA. 1984. Vitamin A in animal and human nutrition. In The retinoids. Vol 1. Ed by Sporn MB, Roberts AB, Goodman DS. Academic Press, Orlando. Pp. 281-391.

Van den Berg M. 1998. Toxic equivalency factors (TEFs) for PCBs, PCDDs, PCDFs for humans and wildlife. Environ. Health. Perspect. 106: 775-792.

Vijayan MM, Feist G, Otto DME, Schreck CB, Moon TW. 1997. 3,3',4,4'-tetrachlorobiphenyl affects cortisol dynamics and hepatic function in rainbow trout. Aquatic. Toxicol. 37: 87-98.

Wassermann D, Wassermann M, Cucos S, Djavaherian M. 1973. Function of adrenal gland-zona fasciculata in rats recieving polychlorinated biphenyls. Environ. Res. 6: 334-338.

Weber LW, Lebofsky M, Ŝtahl BU, Gorski JR, Muzi G, Rozman. 1991. Reduced activities of key enzymes of gluconeogenesis as possible cause of 2,3,7,8-tetrachlorbidenzo-p-dioxin (TCDD) in rats. Toxicol. 66: 133-144.

Weerasinghe NCA, Gross ML, Lisk DJ. 1985. Polychlorinated dibenzodioxins and polychlorinated dibenzofurans in sewage sludges. Chemosphere. 14: 557-562.

Westerhof I, Mol JA, Van den Brom WE, Lumeij JT, Rijnberk A. 1994. Diurnal rhythms in plasma concentrations of corticosterone in racing pigeons exposed to different light regimens, and the influence of frequent blood sampling. Avian. Dis. 38: 428-434.

Wingfield JC, Matt KS, Farner DS. 1984. Physiological properties of steroid hormone-binding proteins in avian blood. Gen. Compar. Endocrin. 53: 281-292.

Wingfield JC. 1999. Modulation of the adrenocortical response to stress in birds. Persp. Comp. Endocrin. 529-528.

Won HT, Murvihill MJ, Wakeford BJ. 2001. Multiresidue methods for the determination of chlorinated pesticides and polychlorinated biphenyls (PCBs) in wildlife tissues by Gas chromatography/Mass spectrometry. Technical Report Series No.335E. Canadian Wildlife Service, Headquarters, Hull, Quebec, Canada.

Zach R, Mayoh KR. 1982. Weight and feather growth of nestling tree swallows. Can. J. Zool. 60: 1080-1090.

Zile MH. 1992. Vitamin A homeostasis endangered by environmental pollutants. Proc. Soc. Exp. Med. 201:141-153.

APPENDIX

Table 1. List of organochlorine contaminants

1,2,4,5-tetrachlorobenzene (TClBz), 1,2,3,4-TClBZ, PnClBz, α–hexachlorocyclohexane (HCH), hexachlorobenzene (HCB), β–HCH, γ-HCB, octachlorostyrene (OCS), heptachlor (HE), oxychlordane, t-chlordane, c-chlordane, t-nonachlor, p,p'-DDE, dieldrin, p,p'-DDD, c-nonachlor, p,p'-dichloro diphenyltrichloroethane (DDT), photomirex, mirex, tris-chlorophenol methanol (TCPM)

Table 2. List of PCBs measured in insect and sediment samples

PCB 1, 3, 4-10, 7-10, 6, 8-5, 19, 12-13, 54-29, 26, 25, 31-28, 50, 33-20, 53, 45, 51, 22, 46, 52, 43, 49, 47-48, 44, 59, 42, 71-41, 40, 100, 63, 73, 70-76-98, 66, 95, 91, 81-87, 85, 136, 110, 82, 151, 138, 172, 16/32, 70/76, 149, 178, 180, 31, 95, 118, 187, 170/190, 28, 66, 146, 183, 201, 33/20, 56/60, 153, 128, 206, 22, 22, 92, 105, 174, 196, 52, 101/90, 179, 177, 203, 49, 99, 141, 202, 208, 47/48, 97, 130, 171, 195, 44, 87, 176, 156, 207, 42, 85, 137, 200, 194 (International Union of Pure and Applied Chemistry IUPAC#)(Ballschmiter and Zell 1980).

Table 3. Means and standard deviations of p,p' DDE, chlorinated pesticides, total polychlorinated biphenyls (µg/g ww), and total mercury (µg/g dw) with % coefficient of variation in 16 day old tree swallow nestlings

1999

	P, P' DDE		CHC[a]		total PCB[b]		Hg[c]	%CV	%Lipid	%Moisture
SITE (*)	Mean	SD.	Mean	S.D.	Mean	SD		(PCB)		
UC Bird Sanctuar (c)	0.051	0.081	0.047	0.075	0.09	0.03	0.16	38	7.2	72
Grasse River N (c)	0.015	0.00	0.020	0.00	10.41	0.00	0.11	0	9.7	66
Cornwall Island (c)	0.049	0.021	0.132	0.246	1.95	1.94	0.05	99	12.5	68
Hoasic Creek (r)	0.013	0.004	0.014	0.005	0.03	0.01	0.13	61	8.5	69
Cooper Marsh (r)	0.025	0.005	0.049	0.065	0.21	0.06	0.13	32	9.1	69
Turtle Creek (c)	0.100	0.119	0.109	0.113	29.80	16.56	0.07	55	10.9	71
Grasse River S (c)	0.017	0.009	0.020	0.015	31.70	17.07	0.09	54	13.9	64
Sewage Treatment (c)	0.050	0.039	0.056	0.035	0.37	0.29	0.09	80	7.1	69

* c - contaminated site
* r - reference site
CHC[a] - total sum chlorinated pesticides (see Chapter 1 methods for congener names).
PCB[b] - total sum polychlorinated biphenyls (see Chapter 1 methods for congener names).
Hg[c] - total mercury (dw) (includes organic and inorganic mercury).
% CV - coefficient of variation (Standard deviation/mean)
%lipid - % lipid of 16 day old tree swallow chicks taken during contaminant analysis
% moisture - % moisture of 16 day old tree swallow chicks taken during contaminant analysis

Table 4. Means and standard deviations of p,p' DDE, chlorinated pesticides, and total polychlorinated biphenyls (ug/g ww), total mercury (ug/g dw) with % coefficient of variation, % lipid and % moisture in 16 day old tree swallow nestlings, n=65.

2000

SITE (*)	P,P'-DDE		CHC		PCB		Hg	%CV	%Lipid	%Moisture
	Means	SD	Means	SD	Means	SD		(PCB)		
Grasse River N (c)	0.033	0.011	0.039	0.016	58.64	9.91	0.08	7	8.7	NA
Cornwall Island (c)	0.026	0.011	0.061	0.025	1.59	0.79	0.05	49	9.7	67.8
Hoasic Creek (r)	0.012	0.006	0.015	0.007	0.01	0.01	0.20	74	8.1	69.5
Cooper Marsh (r)	0.014	0.006	0.024	0.006	0.22	0.03	0.06	17	8.5	60.8
Turtle Creek (c)	0.031	0.006	0.049	0.007	51.59	23.98	0.04	46	10.2	64.7
Grasse River S (c)	0.023	0.009	0.026	0.010	69.13	39.40	0.08	7	7.6	NA
Sewage Treatment (c)	0.030	0.005	0.049	0.008	0.31	0.04	0.04	12	9.0	69.4
Raquette River (c)	0.016	0.003	0.019	0.004	2.10	1.37	0.09	65	9.5	67.8

* c - contaminated site
* r - reference site
CHC - total chlorinated pesticides (see Chapter 1)
PCB - total PCB sum of individual congeners (exlcuding non-ortho PCBs) (see Chapter 1)
% CV - coefficient of variation (Standard deviation/mean)
%lipid - % lipid of 16 day old tree swallow chicks taken during contaminant analysis
% moisture - % moisture of 16 day old tree swallow chicks taken during contaminant analysis

Table 5. Mean basal, stress corticosterone (ng/ml), body weight (g), and % lipid of 16 d old tree swallows collected from the St. Lawrence river.

<u>1999</u>

SITE (*)	CORTA[b] Mean	SD	B_A[a] Mean	SD	BWT Mean	SD	%LIPID Mean	SD
UC bird sanctuary (c)	20.31	3.04	47.02	16.30	19.74	1.13	7.2	0..36
Grasse River N (c)	4.00	2.82	42.77	55.33	19.75	1.76	9.7	0.00
Cornwall Island (c)	12.61	15.78	14.21	8.02	20.09	1.08	12.5	0.91
Hoasic Creek (r)	37.40	15.88	16.05	10.39	20.24	1.97	8.5	0.15
Cooper Marsh (r)	12.15	16.21	18.80	23.48	19.54	1.68	9.1	0.33
Turtle Creek (c)	9.96	8.68	15.80	18.05	19.78	2.46	10.9	1.23
Grasse River S (c)	10.15	4.03	21.21	29.29	19.40	.31	13.9	0.88
Sewage Treatment (c)	6.58	1.82	19.57	29.55	21.07	1.92	7.1	1.00

* c - contaminated site
* r - reference site
B_A[a] - corticosterone (ng/ml) at 10 minute stress interval minus basal corticosterone (ng/ml) (time=0 min)
CORTA[b] - corticosterone (ng/ml) at basal level (time 0 sampling time)
Sample sizes; Upper Canada Bird Sanctuary (7), Hoasic Creek (3), Grasse River N (2), Grasse River S (3), Turtle Creek (7), Cooper Marsh (8), Cornwall Sewage Treatment Plant (8), Cornwall Island (10).
BWT - mass of 16 day old tree swallow chick
% Lipid - % lipid taken from 16 day tree swallow chick during contaminant analysis

Table 6. Mean basal, 10 min stress corticosterone (ng/ml), body weight (g), and % lipid of 16 d old tree swallows collected from the St. Lawrence river.

2000

SITE (*)	CORTA[b] Mean	SD	B_A[a] Mean	SD	BWT Mean	SD	%LIPID Mean	SD
Grasse River N (c)	5.74	2.21	68.38	26.15	19.94	.96	8.7	.904
Cornwall Island (c)	10.47	18.33	57.29	24.95	21.56	1.61	9.7	.655
Hoasic Creek (r)	20.78	13.29	75.59	56.92	20.52	2.90	8.1	.918
Cooper Marsh (r)	16.47	25.67	62.27	29.82	22.21	1.35	8.5	.464
Turtle Creek (c)	10.49	10.37	98.76	60.25	21.82	1.31	10.2	.525
Grasse River S (c)	3.40	2.93	57.58	26.86	21.90	1.69	7.6	.636
Sewage Treatment (c)	6.82	10.47	71.17	42.52	22.89	1.33	9.0	.763
Raquette River (c)	16.82	22.59	83.77	57.26	20.68	3.06	9.5	.835

* c - contaminated site
* r - reference site
B_A[a] - corticosterone (ng/ml) at 10 minute stress interval minus basal corticosterone (ng/nl) (time=0 min)
CORTA [b]- corticosterone (ng/ml) at basal level (time 0 sample)
Sample sizes; Raquette River (10), Hoasic Creek (10), Grasse River N (4), Grasse River S (3), Turtle Creek (10), Cooper Marsh (10), Cornwall Sewage Treatment Plant (10), Cornwall Island (7).
BWT - mass of chick at 16 day old
% Lipid - lipid extracted from 16 day old tree swallow for contaminant analysis

RAW DATA

yr	site	bird	hcb	ocs	he	oxy	nonachlo	dde
1999	cooper	j02	0.003	0	0.003	0	0.001	0.161
1999	cooper	j03	0.002	0	0.001	0	0.001	0.018
1999	cooper	j06	0.001	0	0.001	0	0	0.024
1999	cooper	j09	0.00001	0	0	0	0	0.019
1999	cooper	j11	0.002	0	0.001	0	0.00001	0.015
1999	cooper	j12	0.002	0	0.002	0	0.00001	0.027
1999	cooper	j13	0.00001	0	0.001	0	0.00001	0.014
1999	cooper	j16	0.001	0	0.001	0	0.00001	0.019
1999	cooper	j18	0.001	0	0.00001	0	0	0.024
1999	cooper	j20	0.001	0	0.00001	0	0.00001	0.023
1999	cooper	j22	0.001	0	0.002	0	0.001	0.015
1999	corn	c03	0.001	0	0.002	0.004	0.002	0.017
1999	corn	c04	0.003	0	0.004	0.004	0.002	0.028
1999	corn	c09	0.002	0	0.006	0.004	0.005	0.059
1999	corn	c10	0.003	0.001	0.004	0.002	0.002	0.039
1999	corn	c11	0.008	0	0.01	0.02	0.00001	0.798
1999	corn	c12	0.003	0	0.004	0.002	0.002	0.031
1999	corn	c13	0.002	0	0.003	0.002	0.002	0.023
1999	corn	c14	0.002	0	0.003	0.002	0.002	0.024
1999	corn	c15	0.002	0	0.003	0.004	0.003	0.018
1999	gras n	al10	0.001	0	0	0.001	0	0.001
1999	gras s	ny06	0.00001	0	0	0	0	0.011
1999	gras s	ny08	0.00001	0	0	0	0	0.013
1999	gras s	ny21	0.00001	0	0	0	0	0.011
1999	hoasic c	h10	0	0	0	0	0	0.016
1999	hoasic c	h13	0	0	0	0	0	0.008
1999	hoasic c	h18	0	0	0	0	0	0.017
1999	stp	p01	0.003	0	0.002	0.001	0.002	0.021
1999	stp	p02	0.003	0	0.002	0	0.002	0.025
1999	stp	p03	0.005	0	0.003	0.002	0.003	0.029
1999	stp	p04	0.004	0	0.006	0.01	0.012	0.061
1999	stp	p05	0.003	0	0.002	0.00001	0.001	0.023
1999	stp	p10	0.006	0	0.002	0.001	0.002	0.022
1999	stp	p15	0.005	0	0.002	0.001	0.002	0.022
1999	stp	p18	0.006	0	0.002	0.001	0.003	0.031
1999	turtle cr	m01	0.013	0	0.008	0.021	0.002	0.489
1999	turtle cr	m17	0.001	0	0.002	0	0.002	0.026
1999	turtle cr	m18	0.003	0	0.006	0.009	0.016	0.051
1999	turtle cr	m20	0.002	0	0.003	0.003	0.022	0.011
1999	turtle cr	m24	0.004	0.003	0.002	0.003	0.029	0.01
1999	turtle cr	m25	0.005	0	0.013	0.016	0.011	0.229
1999	ucbs	u03	0	0	0	0	0	0.012
1999	ucbs	u06	0.00001	0	0.00001	0	0	0.015
1999	ucbs	u08	0.00001	0	0.00001	0	0	0.021

1999 ucbs	u12	0	0	0	0	0	0.014
1999 ucbs	u13	0	0	0	0	0	0.021
1999 ucbs	u16	0.00001	0	0.00001	0	0	0.024
2000 cooper	j03	0.001	0	0.001	0.00001	0.019	0.002
2000 cooper	j04	0.00001	0	0	0.00001	0.009	0.001
2000 cooper	j08	0.001	0	0.001	0.00001	0.013	0.019
2000 cooper	j09	0.001	0	0	0.00001	0.019	0.002
2000 cooper	j12	0.001	0	0	0.00001	0.016	0.002
2000 cooper	j15	0.001	0	0.001	0.00001	0.019	0.002
2000 cooper	j19	0	0	0	0.001	0	0.026
2000 cooper	j20	0.00001	0	0.001	0.00001	0.014	0.00001
2000 cooper	j22	0.001	0	0	0.001	0.016	0.00001
2000 corn	c03	0.003	0.00001	0.001	0.001	0.00001	0.015
2000 corn	c04	0.002	0.00001	0.00001	0.002	0.002	0.00001
2000 corn	c05	0.001	0.00001	0.003	0.004	0.002	0.019
2000 corn	c06	0.004	0.00001	0.003	0.002	0.002	0.021
2000 corn	c07	0.003	0.001	0.001	0.003	0.002	0.003
2000 corn	c08	0.004	0.002	0.002	0.005	0.004	0.004
2000 corn	c09	0.003	0.001	0.001	0.004	0.002	0.002
2000 gras n	ny03	0.001	0	0.00001	0.002	0.002	0.033
2000 gras n	ny09	0.001	0	0.00001	0.003	0.002	0.005
2000 gras n	ny15	0.00001	0	0.00001	0.00001	0.00001	0.03
2000 gras n	ny19	0.00001	0	0.00001	0.00001	0.001	0.022
2000 gras s	al02	0.001	0	0.00001	0.001	0.001	0.022
2000 gras s	al15	0.001	0	0.00001	0.002	0.002	0.033
2000 gras s	al6	0.00001	0	0.00001	0.001	0.002	0.014
2000 hoasic c	h01	0.00001	0	0.00001	0.00001	0.00001	0.006
2000 hoasic c	h05	0.00001	0	0.00001	0.00001	0.00001	0.008
2000 hoasic c	h07	0.00001	0	0.00001	0.00001	0.00001	0.006
2000 hoasic c	h09	0.00001	0	0.00001	0.001	0.00001	0.003
2000 hoasic c	h11	0.00001	0	0.00001	0.00001	0.00001	0.014
2000 hoasic c	h12	0.00001	0	0.00001	0.001	0.00001	0.02
2000 hoasic c	h16	0.00001	0	0.00001	0.00001	0.00001	0.018
2000 hoasic c	h17	0.00001	0	0.00001	0.00001	0.00001	0.017
2000 hoasic c	h18	0.00001	0	0.00001	0.00001	0.00001	0.014
2000 hoasic c	h19	0.00001	0	0.00001	0.00001	0.00001	0.008
2000 hoasic c	h20	0.00001	0	0.00001	0.001	0.00001	0.006
2000 raquette	r02	0.001	0	0.00001	0.001	0.00001	0.016
2000 raquette	r04	0.00001	0	0.00001	0.00001	0.00001	0.018
2000 raquette	r05	0.00001	0	0.00001	0.00001	0.00001	0.014
2000 raquette	r09	0.00001	0	0.00001	0.00001	0.00001	0.013
2000 raquette	r10	0.002	0	0.00001	0.00001	0.001	0.021
2000 raquette	r11	0.00001	0	0.00001	0.001	0.00001	0.014
2000 raquette	r14	0.002	0	0.00001	0.001	0.001	0.016
2000 raquette	r15	0.00001	0	0.00001	0.00001	0.00001	0.01
2000 raquette	r16	0.001	0	0.00001	0.00001	0.00001	0.023

2000 raquette	r18	0.002	0	0.00001	0.00001	0.001	0.017
2000 stp	p01	0.003	0	0.003	0.002	0.002	0.036
2000 stp	p02	0.003	0	0.003	0.002	0.001	0.024
2000 stp	p04	0.004	0	0.003	0.002	0.001	0.032
2000 stp	p05	0.002	0	0.003	0.002	0.001	0.024
2000 stp	p06	0.004	0	0.003	0.002	0.001	0.038
2000 stp	p09	0.003	0	0.002	0.003	0.001	0.026
2000 stp	p10	0.004	0	0.003	0.002	0.002	0.034
2000 stp	p12	0.004	0	0.003	0.002	0.002	0.027
2000 stp	p13	0.003	0	0.003	0.002	0.001	0.03
2000 stp	p17	0.004	0	0.003	0.003	0.002	0.038
2000 turtle cr	m03	0.002	0.00001	0.001	0.002	0.002	0.024
2000 turtle cr	m05	0.002	0.00001	0.003	0.00001	0.003	0.025
2000 turtle cr	m06	0.002	0.00001	0.003	0.001	0.003	0.029
2000 turtle cr	m16	0.002	0.00001	0.002	0.001	0.002	0.029
2000 turtle cr	m20	0.002	0.001	0.002	0.002	0.002	0.03
2000 turtle cr	m25	0.002	0.00001	0.002	0.001	0.002	0.03
2000 turtle cr	m30	0.002	0.00001	0.003	0.002	0.003	0.034
2000 turtle cr	m31	0.002	0.00001	0.003	0.002	0.003	0.029
2000 turtle cr	m35	0:003	0.001	0.004	0.002	0.003	0.042

0	0.001	0	0	0	0	0.00001	0.00001	0.001
0	0.002	0	0	0	0.00001	0	0	0.00001
0	0	0	0	0	0.00001	0	0	0
0.00001	0	0	0	0	0	0	0	0.001
0.00001	0	0	0	0.001	0	0	0	0.00001
0.00001	0	0	0	0.002	0	0	0	0.00001
0.00001	0	0	0	0.001	0	0	0	0.001
0.00001	0	0	0	0.002	0	0	0	0.001
0.00001	0	0	0	0.001	0	0	0	0.002
0.00001	0	0	0	0.001	0	0	0	0.002
0.00001	0	0	0	0	0	0	0	0.001
0.00001	0	0	0	0.002	0	0	0	0.001
0.003	0.00001	0.00001	0.00001	0.002	0	0.00001	0.006	0.064
0.02	0.058	0.00001	0.004	0.002	0	0.001	0.00001	0.007
0.004	0.00001	0.00001	0.001	0.002	0	0.008	0.011	0.019
0.009	0.00001	0.00001	0.002	0.004	0.002	0.009	0.0115	0.038
0.03	0.011	0.001	0.003	0.003	0	0.005	0.013	0.046
0.048	0.01	0.002	0.004	0.004	0	0.006	0.013	0.038
0.034	0.018	0.002	0.003	0.004	0	0	0.001	0.018
0.00001	0.00001	0.00001	0.00001	0.00001	0.915	7.801	8.683	3.434
0.004	0.001	0.00001	0.00001	0.00001	1.836	1.731	4.165	2.789
0.00001	0.00001	0.00001	0.00001	0.00001	0.443	4.053	3.483	2.368
0.001	0.00001	0.00001	0.00001	0.00001	0.184	4.909	6.271	2.175
0.00001	0.00001	0.00001	0.00001	0.00001	0.739	4.788	5.843	2.428
0	0.00001	0.00001	0.00001	0.00001	0	14.107	13.408	5.499
0.00001	0.00001	0.00001	0.00001	0.00001	0.457	3.936	3.843	2.316
0.005	0	0	0	0	0	0	0	0
0.00001	0	0	0	0	0	0	0	0
0.004	0	0	0	0	0	0	0	0
0.001	0	0	0	0	0	0	0	0
0.00001	0	0	0	0	0	0	0	0
0.00001	0	0	0	0	0	0	0	0
0.009	0	0	0	0	0	0	0	0
0.003	0	0	0	0	0	0	0	0
0.002	0	0	0	0	0	0	0	0
0.00001	0	0	0	0	0	0	0	0
0.00001	0	0	0	0	0	0	0	0
0.001	0.023	0.001	0.00001	0.002	0.00001	0.004	0.007	0.055
0.001	0.018	0.001	0.00001	0.002	0.002	0.009	0.008	0.037
0	0.016	0	0.00001	0.002	0	0	0	0.002
0	0.013	0	0.00001	0.00001	0	0.00001	0.004	0.159
0	0.021	0	0.00001	0.002	0.004	0.016	0.019	0.084
0	0.014	0	0.00001	0.002	0	0.00001	0.002	0.177
0.001	0.016	0.001	0.00001	0	0.001	0.009	0.017	0.038
0	0.01	0	0.00001	0.00001	0.002	0.015	0.015	0.039
0	0.021	0	0.00001	0.001	0.00001	0	0.00001	0.086

0.001	0.017	0.001	0.00001	0.001	0.006	0.02	0.015	0.063
0.003	0.00001	0.00001	0.002	0.004	0	0	0.00001	0.00001
0.005	0.001	0.00001	0.001	0.003	0	0	0.00001	0.001
0.003	0.00001	0.00001	0.001	0.003	0	0	0.00001	0.001
0.004	0.00001	0.00001	0.00001	0.001	0	0	0.00001	0.00001
0.005	0.001	0.00001	0.001	0.003	0	0.001	0.00001	0.048
0.004	0.00001	0.00001	0.001	0.002	0	0	0.00001	0.002
0.005	0.00001	0.00001	0.001	0.002	0	0.00001	0.00001	0.002
0.003	0.00001	0.00001	0.001	0.004	0	0	0.00001	0.004
0.004	0.00001	0.00001	0.001	0.003	0	0	0.002	0.001
0.005	0.001	0.00001	0.002	0.005	0	0	0.00001	0.00001
0.004	0.00001	0.00001	0.001	0.002	0.01	0.039	0.041	0.9
0.00001	0	0.00001	0.002	0.003	0.027	0.225	0.36	1.074
0.009	0.001	0.00001	0.002	0.003	0.013	0.068	0.181	1.453
0.002	0.00001	0.00001	0.001	0.003	0.005	0.018	0.054	0.587
0.007	0.00001	0.00001	0.002	0.003	0.003	0.04	0.062	1.775
0.006	0.002	0.00001	0.001	0.002	0.169	1.208	0.997	2.053
0.006	0.00001	0.00001	0.002	0.003	0.015	0.069	0.173	1.775
0.012	0.00001	0.00001	0.001	0.003	0.139	0.833	0.922	0.322
0.012	0.002	0.00001	0.003	0.004	0.024	0.421	0.484	0.323

28	33-20	22	52	49	47-48	44	42	64
0.008	0.001	0.001	0.017	0.017	0.013	0.006	0.009	0.01
0.004	0.00001	0.00001	0.008	0.006	0.009	0.003	0.004	0.006
0.003	0	0	0.004	0.003	0.002	0.00001	0.001	0.002
0.003	0	0	0.004	0.002	0.002	0.00001	0.00001	0.002
0.002	0	0	0.004	0.002	0.003	0	0.001	0.002
0.002	0.00001	0	0.004	0.002	0.003	0	0.00001	0.002
0.002	0.00001	0	0.004	0.002	0.003	0	0.00001	0.002
0.002	0.00001	0	0.004	0.003	0.004	0	0	0.003
0.003	0	0	0.006	0.004	0.003	0.001	0.001	0.003
0.002	0.00001	0	0.005	0.003	0.003	0.001	0.002	0.003
0.002	0.00001	0	0.004	0.003	0.003	0	0.001	0.002
0.075	0.00001	0.00001	0.09	0.122	0.153	0.012	0.022	0.067
0.01	0.00001	0.001	0.064	0.143	0.168	0.014	0.037	0.053
0.061	0.00001	0.007	0.526	0.358	0.185	0.162	0.155	0.329
0.025	0.00001	0.00001	0.047	0.041	0.051	0.002	0.002	0.017
0.006	0.013	0	0	0.063	0.014	0.031	0	0
0.075	0.00001	0.00001	0.033	0.03	0.035	0.002	0.003	0.016
0.429	0.00001	0.00001	0.019	0.015	0.015	0.003	0.004	0.009
0.008	0.00001	0.00001	0.017	0.013	0.015	0.004	0.006	0.009
0.015	0.00001	0.00001	0.02	0.016	0.018	0.006	0.007	0.011
0.247	0.025	0.018	0.885	0.885	0.904	0.174	0.271	0.302
3.076	0.353	0.575	4.04	3.107	2.03	2.265	2.09	2.519
1.503	0.209	0.287	2.393	1.961	1.448	1.237	1.14	1.345
0.395	0.033	0.027	1.568	1.511	1.405	0.403	0.49	0.402
0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0
0.006	0	0	0.009	0.005	0.006	0	0	0.002
0.009	0.00001	0.011	0.009	0.007	0.005	0.005	0.007	0.006
0.004	0.00001	0.00001	0.008	0.005	0.006	0.001	0.002	0.004
0.0001	0.001	0	0.006	0.001	0.002	0	0	0.002
0.004	0.00001	0.00001	0.006	0.004	0.003	0.002	0.002	0.004
0.003	0.00001	0.00001	0.005	0.004	0.005	0.002	0.002	0.004
0.008	0.001	0.001	0.013	0.012	0.01	0.005	0.007	0.008
0.003	0.00001	0.00001	0.006	0.005	0.003	0.002	0.002	0.004
1.903	0.006	2.787	1.942	2.533	0.145	0.168	0.811	2.375
0.833	1.41	0.025	0.062	1.654	1.676	2.558	0.501	0.664
2.048	0.018	0.011	2.715	3.2	4.338	0.182	0.283	1.773
0.666	0.022	0.052	0.703	0.828	1.092	0.222	0.337	0.465
2.88	3.278	3.935	1.179	1.61	1.99	3.135	2.907	0.3771
0.00001	0.00001	0.007	0.005	0.006	0.002	0.003	0.005	0.009
0	0	0	0	0	0	0	0	0
0.001	0	0	0.002	0.001	0.001	0	0	0.00001
0.001	0	0	0.002	0.001	0.001	0	0	0.00001

0.002	0.00001	0	0.003	0.003	0.003	0.00001	0.002	0.002
0.002	0	0	0.002	0.002	0.002	0.00001	0	0.001
0.001	0	0	0.001	0.00001	0.00001	0	0	0.00001

0.003	0	0	0.006	0.006	0.009	0.001	0.003	0.005
0.002	0	0	0.003	0.003	0.005	0	0.001	0.003
0.002	0	0	0.003	0.003	0.003	0	0.001	0.002
0.003	0	0	0.004	0.004	0.007	0.001	0.002	0.004
0.003	0	0	0.004	0.004	0.007	0	0.001	0.003
0.003	0	0	0.005	0.004	0.007	0	0	0.003
0.005	0	0	0.006	0.006	0.011	0	0.001	0.005
0.002	0	0	0.004	0.003	0.005	0		0.002
0.003	0	0	0.005	0.004	0.006	0	0.003	0.004

0.085	0.001	0.00001	0.184	0.214	0.387	0.007	0.017	0.073
0.014	0	0.00001	0.028	0.024	0.023	0.004	0.001	0.009
0.023	0.001	0.002	0.05	0.046	0.061	0.009	0.014	0.021
0.068	0.002	0.002	0.088	0.108	0.152	0.019	0.033	0.054
0.084	0.001	0.003	0.046	0.103	0.053	0.04	0.04	0.053
0.08	0	0.002	0.028	0.133	0.023	0.018	0.035	0.06
0.053	0	0.00001	0.118	0.042	0.173	0.019	0.003	0.015

1.283	0.125	0.102	6.708	7.512	8.099	0.861	1.951	1.058
1.238	0.065	0.048	7.312	7.609	7.084	0.813	1.74	1.211
0.72	0.132	0.134	5.11	5.342	4.684	0.921	1.632	0.809
0.524	0.118	0.076	5.595	5.955	5.953	0.704	1.544	0.776

1.106	0.121	0.111	4.557	4.986	6.059	0.9	1.568	1.114
2.609	0.414	0.383	9.901	10.913	12.113	2.422	3.969	2.155
1.047	0.128	0.107	3.778	4.146	4.558	0.822	1.333	0.848

0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0
0	0	0	0.002	0.001	0	0	0	0
0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0

0.046	0.001	0.002	0.107	0.097	0.069	0.019	0.027	0.051
0.034	0.003	0.004	0.08	0.067	0.05	0.031	0.032	0.041
0.017	0	0	0.064	0.03	0.015	0.00001	0.001	0.01
0.357	0.004	0.001	0.691	0.41	0.213	0.029	0.04	0.266
0.057	0.002	0.003	0.139	0.131	0.102	0.03	0.038	0.066
0.204	0.002	0.001	0.274	0.183	0.31	0.01	0.008	0.063
0.053	0.002	0.004	0.08	0.075	0.09	0.023	0.032	0.045
0.032	0.003	0.004	0.07	0.061	0.047	0.022	0.026	0.036
0.092	0.00001	0	0.185	0.156	0.103	0.003	0.003	0.055

0.059	0.004	0.005	0.12	0.104	0.076	0.036	0.042	0.063
0.003	0	0.00001	0.006	0.003	0.003	0.01	0.00001	0.002
0.003	0	0.00001	0.005	0.003	0.003	0.00001	0.001	0.003
0.005	0	0.00001	0.006	0.005	0.005	0.00001	0.00001	0.004
0.003	0	0	0.004	0.003	0.003	0	0.001	0.002
0.006	0	0.00001	0.015	0.014	0.012	0.004	0.004	0.007
0.003	0	0.00001	0.006	0.008	0.129	0.00001	0.00001	0.004
0.005	0	0	0.006	0.005	0.005	0.002	0.003	0.004
0.005	0.003	0.00001	0.006	0.008	0.008	0.002	0.001	0.004
0.004	0	0.00001	0.006	0.004	0.004	0.001	0.001	0.003
0.005	0	0.00001	0.006	0.004	0.004	0.00001	0.001	0.004
4.539	0.013	0.007	3.721	5.188	6.439	0.247	0.37	2.074
2.16	0.033	0.114	2.148	2.481	3.075	0.651	1.027	1.34
2.102	0.029	0.047	2.58	2.577	3.098	0.602	0.792	1.535
1.504	0.013	0.016	1.876	2.267	2.779	0.165	0.273	0.948
0.723	0.15	0.025	0.885	0.92	1.166	0.157	0.27	0.471
3.77	0.14	0.296	4.631	4.688	5.422	1932	2.371	2.734
1.985	0.022	0.049	2.234	2.483	3.058	0.454	0.762	1.312
2.829	0.023	0.251	2.841	3.18	4.604	1.107	1.481	1.859
3.316	0.069	0.15	3.855	4.426	5.555	1.09	1.753	2.414

74	70-76	95	66	56-60	92	101-90	99	97
0.013	0.016	0.002	0.038	0.008	0.003	0.013	0.013	0.004
0.008	0.009	0.002	0.023	0.006	0.003	0.01	0.009	0.003
0.003	0.004	0.001	0.009	0.002	0.002	0.007	0.003	0.002
0.003	0.003	0.01	0.009	0.001	0.002	0.006	0.003	0.00001
0.002	0.003	0.001	0.004	0.001	0.003	0.009	0.005	0.001
0.004	0.004	0.001	0.011	0.002	0.002	0.006	0.005	0.00001
0.003	0.003	0.001	0.012	0.002	0.002	0.006	0.005	0.001
0.004	0.003	0.001	0.013	0.002	0.002	0.007	0.005	0.001
0.004	0.005	0.002	0.013	0.00001	0.003	0.009	0.004	0.002
0.004	0.005	0.001	0.013	0.003	0.002	0.005	0.005	0.001
0.002	0.003	0.00001	0.009	0.002	0.002	0.004	0.003	0.001
0.164	0.069	0.013	0.395	0.078	0.026	0.116	0.102	0.028
0.207	0.034	0.013	0.326	0.092	0.036	0.158	0.144	0.051
0.312	0.433	0.065	0.986	0.202	0.048	0.194	0.131	0.074
0.031	0.027	0.009	0.088	0.013	0.015	0.035	0.026	0.004
0.008	0.01	0.008	0.007	0.045	0.009	0.034	0.033	0.02
0.036	0.022	0.007	0.092	0.018	0.014	0.039	0.029	0.005
0.01	0.011	0.005	0.032	0.005	0.008	0.017	0.012	0.005
0.01	0.011	0.004	0.031	0.006	0.008	0.016	0.01	0.005
0.012	0.014	0.004	0.037	0.008	0.008	0.019	0.013	0.006
0.155	0.13	0.127	0.303	0.078	0.251	0.308	0.134	0.066
2.328	2.99	0.495	4.963	2.17	0.42	1.571	0.621	0.559
1.037	1.43	0.289	2.455	0.947	0.295	0.837	0.318	0.262
0.262	0.217	0.215	0.479	0.116	0.357	0.483	0.231	0.106
0	0	0	0.002	0	0	0.001	0.001	0
0	0	0	0.00001	0	0	0.00001	0.00001	0
0	0	0	0.002	0	0	0.001	0.00001	0
0.009	0.004	0.001	0.037	0.005	0.005	0.016	0.013	0.0001
0.009	0.003	0.02	0.006	0.004	0.012	0.005	0.004	0.006
0.006	0.006	0.002	0.023	0.004	0.045	0.013	0.009	0.002
0.002	0.00001	0.001	0.014	0.004	0.009	0.014	0.006	0.0001
0.004	0.006	0.002	0.014	0.003	0.003	0.009	0.004	0.002
0.004	0.005	0.002	0.014	0.003	0.004	0.008	0.007	0.002
0.01	0.011	0.002	0.029	0.006	0.004	0.01	0.011	0.004
0.005	0.001	0.012	0.003	0.002	0.007	0.004	0.002	0.004
1.524	0.364	6.266	1.129	0.368	1.394	1.16	0.138	0.667
1.04	1.677	1.295	0.193	3.61	0.874	0.2	0.964	0.856
2.844	2.338	0.311	5.776	1.084	0.412	1.692	1.676	0.299
0.836	0.593	0.094	1.729	0.5	0.114	0.478	0.408	0.17
5.679	2.207	0.41	1.925	1.077	0.8	1.2	0.803	1.711
0.008	0.001	0.025	0.006	0.002	0.005	0.006	0.001	0.003
0	0.001	0.001	0	0.004	0	0	0.002	0.002
0.002	0.00001	0.00001	0.004	0.00001	0.00001	0.004	0	0.001
0.002	0.002	0.00001	0.006	0.00001	0.001	0.004	0.00001	0.002

0.003	0.003	0.00001	0.009	0.002	0.0001	0.003	0.003	0.00001
0.003	0.003	0.00001	0.008	0.001	0.001	0.004	0.003	0.001
0.001	0.001	0.00001	0.004	0.00001	0.00001	0.001	0.00001	0.001
0.006	0.005	0.002	0.019	0.004	0.004	0.01	0.008	0.002
0.003	0.004	0	0.011	0.002	0.002	0.006	0.004	0.001
0.003	0.003	0.001	0.011	0.002	0.003	0.006	0.005	0.001
0.005	0.005	0.001	0.015	0.004	0.003	0.008	0.006	0.002
0.005	0.005	0.001	0.016	0.003	0.003	0.008	0.007	0.001
0.005	0.005	0.001	0.016	0.002	0.003	0.008	0.007	
0.007	0.007	0.001	0.022	0.004	0.004	0.01	0.008	0.001
0.004	0.004	0	0.013	0.002	0.003	0.007	0.006	
0.005	0.005	0.001	0.017	0.005	0.003	0.008	0.006	0.002
0.111	0.055	0.023	0.229	0.035	0.044	0.092	0.079	0.013
0.018	0.015	0.005	0.047	0.008	0.011	0.024	0.017	0.002
0.033	0.022	0.007	0.061	0.016	0.015	0.033	0.025	0.009
0.09	0.048	0.012	0.175	0.034	0.019	0.06	0.059	0.017
0.097	86	0.017	0.214	0.047	0.021	0.07	0.057	0.025
0.11	0.06	0.021	0.214	0.05	0.028	0.087	0.071	0.027
0.033	0.021	0.009	0.088	0.012	0.015	0.034	0.027	0.003
0.624	0.372	0.844	1.277	0.255	1.826	1.369	0.857	0.186
0.747	0.338	0.832	1.13	0.215	1.942	1.58	0.862	0.232
0.603	0.288	0.62	0.926	0.255	1.266	1.202	0.615	0.21
0.749	0.205	0.719	0.735	0.202	1.473	1.089	0.511	0.164
0.675	0.348	0.653	0.963	0.248	1.079	1.1	0.792	0.206
1.303	0.801	1.4	1.742	0.506	2.168	2.2	1.295	0.404
0.615	0.381	0.51	0.914	0.226	0.707	0.939	0.592	0.193
0	0	0	0.002	0	0	0	0	0
0	0	0	0.001	0	0	0	0	0
0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0.001	0	0
0	0	0	0.002	0	0	0	0	0
0	0	0	0.002	0	0	0.001	0	0
0	0	0	0.002	0	0	0	0	0
0	0	0	0.002	0	0	0	0	0
0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0.002	0	0
0.067	0.062	0.012	0.15	0.024	0.017	0.054	0.039	0.014
0.048	0.048	0.01	0.102	0.024	0.014	0.041	0.029	0.013
0.019	0.016	0.007	0.076	0.013	0.016	0.036	0.016	0.00001
0.313	0.267	0.093	1.11	0.208	0.067	0.242	0.126	0.036
0.069	0.068	0.018	0.14	0.023	0.024	0.071	0.047	0.02
0.274	0.126	0.024	0.533	0.083	0.033	0.128	0.104	0.01
0.061	0.058	0.014	0.144	0.037	0.02	0.068	0.04	0.023
0.042	0.039	0.01	0.083	0.021	0.014	0.052	0.033	0.017
0.209	0.088	0.02	0.368	0.049	0.028	0.1	0.078	0.005

0.073	0.017	0.02	0.16	0.033	0.022	0.091	0.052	0.028
0.004	0.005	0.002	0.018	0.003	0.005	0.012	0.006	0.002
0.004	0.005	0.002	0.016	0.002	0.003	0.011	0.006	0.002
0.006	0.007	0.002	0.023	0.003	0.004	0.013	0.009	0.002
0.004	0.005	0.001	0.014	0.003	0.003	0.011	0.007	0.003
0.007	0.007	0.003	0.025	0.004	0.006	0.014	0.009	0.003
0.007	0.006	0.002	0.025	0.003	0.005	0.014	0.009	0.001
0.006	0.008	0.002	0.021	0.004	0.004	0.014	0.009	0.003
0.007	0.007	0.002	0.022	0.003	0.005	0.012	0.009	0.001
0.005	0.005	0.002	0.021	0.003	0.004	0.012	0.007	0.002
0.006	0.006	0.002	0.022	0.003	0.004	0.013	0.009	0.002
7.017	2.586	0.464	11.447	2.229	0.675	3.366	3.141	0.592
2.468	1.701	0.271	4.833	1.253	0.244	1.12	0.904	0.419
2.945	2.027	0.321	6.09	0.886	0.306	1.421	1.098	0.425
2.822	0.958	0.215	5.346	0.955	0.288	1.364	1.182	0.258
1.042	1.586	0.11	2.266	0.458	0.134	0.571	0.338	0.182
4.686	2.79	0.538	10.418	2.765	0.472	2.335	1.874	0.905
2.77	2.551	0.282	5.298	1.196	0.29	1.353	1.107	0.431
3.74	2.171	0.332	6.822	1.783	0.383	1.791	1.523	0.674
5.31	2.551	0.471	9.218	2.418	0.538	2.656	2.251	0.948

87	85	110	151	149	118	146	153	105
0.008	0.006	0.01	0.002	0.005	0.016	0.005	0.016	0.008
0.006	0.005	0.008	0.002	0.004	0.013	0.004	0.014	0.008
0.004	0.003	0.006	0.002	0.004	0.01	0.004	0.016	0.006
0.003	0.003	0.005	0.002	0.005	0.01	0.004	0.015	0.006
0.002	0.003	0.005	0.003	0.006	0.007	0.006	0.006	0.004
0.003	0.003	0.005	0.002	0.004	0.013	0.005	0.017	0.008
0.003	0.003	0.005	0.002	0.004	0.008	0.004	0.012	0.006
0.004	0.004	0.006	0.00001	0.004	0.009	0.004	0.013	0.006
0.004	0.004	0.007	0.002	0.005	0.011	0.004	0.017	0.007
0.002	0.002	0.004	0.002	0.006	0.01	0.005	0.014	0.004
0.003	0.003	0.005	0.002	0.007	0.008	0.005	0.02	0.005
0.066	0.058	0.086	0.009	0.024	0.173	0.019	0.059	0.121
0.093	0.071	0.105	0.013	0.03	0.257	0.028	0.094	0.167
0.122	0.077	0.2	0.027	0.059	0.205	0.036	0.107	0.151
0.018	0.013	0.027	0.012	0.023	0.041	0.023	0.062	0.025
0	0.011	0.003	0.019	0.019	0.047	0.051	0.038	0.099
0.022	0.017	0.032	0.01	0.022	0.062	0.018	0.042	0.041
0.01	0.006	0.015	0.007	0.014	0.021	0.011	0.029	0.013
0.009	0.006	0.014	0.008	0.014	0.018	0.013	0.022	0.012
0.011	0.007	0.016	0.006	0.013	0.022	0.01	0.025	0.015
0.122	0.054	0.288	0.146	0.273	0.249	0.141	0.375	0.091
0.866	0.461	1.573	0.207	0.52	1.464	0.147	0.656	0.958
0.418	0.212	0.852	0.159	0.348	0.715	0.137	0.462	0.408
0.197	0.089	0.543	0.206	0.405	0.4	0.204	0.551	0.141
0	0.001	0.001	0	0.001	0.003	0.002	0.008	0.001
0	0	0	0	0	0.002	0	0.003	0
0	0	0	0	0.001	0.002	0.001	0.005	0
0.005	0.008	0.006	0.003	0.013	0.036	0.021	0.075	0.018
0.004	0.011	0.004	0.008	0.015	0.007	0.024	0.008	0.001
0.006	0.006	0.009	0.004	0.01	0.02	0.012	0.038	0.011
0.005	0.007	0.003	0.004	0.021	0.043	0.04	0.089	0.029
0.004	0.004	0.009	0.003	0.007	0.013	0.006	0.019	0.008
0.004	0.004	0.007	0.004	0.008	0.011	0.008	0.021	0.007
0.007	0.005	0.011	0.003	0.007	0.016	0.005	0.022	0.008
0.003	0.006	0.001	0.004	0.01	0.004	0.014	0.005	0.00001
0.565	0.953	0.126	0.38	1.673	0.196	0.596	1.244	0.025
0.32	0.558	0.397	0.786	0.069	0.168	1.207	0.092	0.32
0.955	0.742	1.428	0.159	0.384	1.918	0.154	0.067	1.331
0.264	0.173	0.352	0.05	0.096	0.566	0.061	0.172	0.382
0.142	0.361	2.5	0.172	0.644	1.654	0.027	0.102	0.051
0.003	0.003	0.005	0	0.002	0.009	0.006	0.018	0.006
0	0.001	0.001	0.001	0	0.001	0.003	0.002	0.006
0.001	0.001	0.001	0.002	0.004	0.003	0.01	0.003	0.00001
0.002	0.003	0.001	0.002	0.007	0.003	0.011	0.004	0.00001

0.002	0.002	0.002	0.00001	0.002	0.005	0.002	0.007	0.003
0.002	0.002	0.003	0.002	0.003	0.005	0.003	0.01	0.003
0.001	0.002	0.00001	0.001	0.005	0.002	0.008	0.002	0
0.006	0.006	0.009	0.003	0.006	0.014	0.006	0.019	0.01
0.003	0.004	0.005	0.002	0.003	0.008	0.004	0.01	0.006
0.003	0.003	0.005	0.002	0.004	0.009	0.005	0.015	0.006
0.004	0.005	0.007	0.002	0.004	0.011	0.005	0.013	0.008
0.004	0.004	0.007	0.002	0.005	0.012	0.006	0.019	0.008
0.004	0.005	0.007	0.003	0.005	0.013	0.006	0.021	0.008
0.005	0.006	0.008	0.002	0.006	0.014	0.006	0.018	0.01
0.004	0.005	0.006	0.002	0.005	0.011	0.005	0.015	0.008
0.005	0.005	0.007	0.002	0.005	0.013	0.006	0.018	0.009
0.056	0.038	0.079	0.023	0.044	0.128	0.03	0.085	0.064
0.01	0.009	0.016	0.008	0.015	0.03	0.011	0.034	0.016
0.018	0.012	0.024	0.009	0.018	0.04	0.015	0.039	0.022
0.037	0.03	0.059	0.01	0.021	0.106	0.017	1.863	0.056
0.044	0.032	0.063	0.015	0.029	0.09	0.019	0.055	0.054
0.051	0.036	0.075	0.019	0.039	0.109	0.021	0.079	0.065
0.015	0.012	0.026	0.013	0.022	0.041	0.018	0.049	0.024
0.538	0.187	1.168	0.909	1.548	0.732	0.661	1.142	0.245
0.584	0.212	1.374	1.004	1.689	0.854	0.724	0.822	0.27
0.437	0.155	1.134	0.692	1.164	0.643	0.488	0.938	0.186
0.435	0.139	0.978	0.782	1.212	0.557	0.526	1	0.17
0.458	0.177	1.163	0.626	1.062	0.726	0.422	0.789	0.226
0.901	0.335	2.51	1.208	2.174	1.393	0.891	1.384	0.434
0.378	0.157	1.094	0.421	0.808	0.624	0.33	0.788	0.194
0	0	0	0	0	0.001	0	0.008	0
0	0	0	0	0	0.001	0	0.002	0
0	0	0	0	0	0	0	0.003	0
0	0	0	0	0	0.001	0	0.003	0
0	0	0	0	0	0.002	0.001	0.003	0
0	0	0	0	0	0.002	0.001	0.003	0.001
0	0	0	0	0	0.001	0	0.008	0
0	0	0	0	0	0	0.002	0.01	0
0	0	0	0	0	0.002	0	0.002	0
0	0	0	0	0	0.001	0	0.006	0
0	0	0	0	0	0.002	0	0.003	0
0.029	0.019	0.049	0.009	0.022	0.077	0.012	0.064	0.035
0.023	0.014	0.037	0.006	0.015	0.052	0.008	0.04	0.026
0.015	0.013	0.021	0.006	0.018	0.058	0.012	0.048	0.03
0.155	0.097	0.282	0.026	0.062	0.302	0.023	0.102	0.217
0.038	0.021	0.066	0.012	0.031	0.082	0.016	0.066	0.04
0.056	0.047	0.071	0.014	0.03	0.177	0.019	0.086	0.089
0.039	0.023	0.065	0.012	0.033	0.087	0.019	0.065	0.049
0.026	0.014	0.044	0.008	0.02	0.065	0.011	0.05	0.028
0.051	0.034	0.071	0.011	0.036	0.265	0.03	0.131	0.152

0.047	0.027	0.078	0.012	0.036	0.105	0.016	0.079	0.052
0.005	0.005	0.009	0.004	0.008	0.017	0.009	0.029	0.01
0.005	0.004	0.008	0.003	0.007	0.014	0.007	0.022	0.008
0.006	0.006	0.01	0.003	0.008	0.018	0.009	0.029	0.011
0.005	0.004	0.008	0.003	0.006	0.014	0.006	0.022	0.007
0.006	0.005	0.009	0.004	0.009	0.016	0.008	0.025	0.009
0.006	0.004	0.009	0.004	0.01	0.019	0.009	0.029	0.01
0.006	0.006	0.011	0.004	0.009	0.018	0.009	0.031	0.011
0.005	0.004	0.009	0.004	0.008	0.016	0.007	0.025	0.009
0.005	0.006	0.011	0.004	0.009	0.016	0.009	0.028	0.01
0.006	0.005	0.009	0.004	0.008	0.018	0.009	0.03	0.01
1.874	1.428	2.1	0.184	0.37	4.698	4.698	1.152	2.876
0.686	0.471	0.945	0.108	0.224	1.331	1.331	0.346	0.849
0.832	0.592	1.158	0.126	0.25	1.736	0.13	0.425	1.077
0.733	0.579	0.927	0.093	0.182	1.904	1.904	0.526	1.155
0.324	0.254	0.425	0.056	0.117	0.754	0.754	0.251	0.425
1.443	1.023	1.886	0.172	0.403	2.629	0.187	0.616	1.806
0.8	0.583	1.097	0.115	0.244	1.743	1.743	0.453	1.096
0.954	0.665	1.216	0.144	0.246	2.231	2.231	0.569	1.199
1.528	1.084	1.982	0.21	0.404	3.493	3.492	0.909	2.079

179	141	130	176	137	158	138	178	187
0.00001	0.00001	0.00001	0	0.001	0.014	0.005	0.002	0.006
0.00001	0.002	0.00001	0	0.001	0.00001	0.014	0.002	0.007
0.00001	0.001	0.00001	0	0.00001	0	0.014	0.002	0.007
0.00001	0.002	0.00001	0	0.001	0.001	0.013	0.002	0.009
0.00001	0.002	0.00001	0	0.001	0.001	0.016	0.003	0.009
0.00001	0.002	0.00001	0	0.001	0.001	0.016	0.002	0.008
0.00001	0.001	0.00001	0	0.001	0.001	0.011	0.001	0.008
0.00001	0.002	0	0	0.001	0.001	0.012	0	0.008
0.00001	0.002	0.00001	0.00001	0.001	0.001	0.014	0.002	0.007
0.00001	0.002	0.00001	0	0.00001	0.002	0.014	0.002	0.006
0.00001	0.003	0.00001	0	0.001	0.001	0.016	0.002	0.008
0.002	0.011	0.005	0.00001	0.009	0.009	0.073	0.009	0.025
0.004	0.017	0.008	0.001	0.009	0.014	0.11	0.012	0.036
0.009	0.168	0.007	0.003	0.01	0.012	0.112	0.026	0.078
0.004	0.007	0.003	0.00001	0.006	0.005	0.056	0.018	0.049
0.027	0.004	0.004	0.005	0.002	0.006	0.009	0.083	0.021
0.003	0.007	0.003	0	0.005	0.005	0.047	0.011	0.029
0.003	0.004	0.002	0.00001	0.00001	0.003	0.028	0.008	0.02
0.003	0.004	0.002	0.001	0.004	0.003	0.025	0.012	0.022
0.002	0.004	0.002	0.00001	0.003	0.003	0.026	0.006	0.019
0.029	0.039	0.012	0.009	0.022	0.024	0.351	0.062	0.244
0.039	0.116	0.031	0.012	0.046	0.064	0.737	0.058	0.224
0.03	0.071	0.02	0.01	0.031	0.039	0.491	0.059	0.228
0.041	0.059	0.019	0.012	0.032	0.039	0.501	0.076	0.31
0	0	0	0	0	0	0.006	0	0.003
0	0	0	0	0	0	0.003	0	0.002
0	0	0	0	0	0	0.003	0	0.002
0	0.005	0.003	0	0.005	0.006	0.057	0.009	0
0.003	0.001	0	0.002	0.002	0.021	0.004	0.012	0.003
0.001	0.001	0.004	0.002	0	0.003	0.003	0.031	0.006
0.0001	0.005	0.005	0.001	0.006	0.007	0.083	0.023	0.086
0.00001	0.002	0.001	0	0.002	0.002	0.019	0.003	0.01
0.00001	0.002	0.00001	0	0.002	0.018	0.002	0.018	0.004
0.00001	0.002	0.00001	0	0.002	0.002	0.018	0.002	0.008
0.002	0.00001	0.00001	0.001	0.001	0.013	0.002	0.006	0.002
0.073	0.037	0.009	0.048	0.065	0.754	0.106	0.332	0.082
0.79	0.016	0.049	0.023	0.004	0.027	0.04	0.406	0.049
0.029	0.085	0.042	0.008	0.063	0.079	0.853	0.144	0.409
0.013	0.03	0.014	0.003	0.017	0.023	0.222	0.039	0.112
0.008	0.057	0.08	0.873	0.078	0.299	0.067	0.153	0.068
0	0	0.00001	0	0.001	0.001	0.015	0.004	0.016
0.002	0	0	0	0	0.00001	0.00001	0.005	0.001
0.001	0.00001	0	0.00001	0.00001	0.008	0.002	0.005	0.001
0.001	0.00001	0	0.001	0.00001	0.009	0.002	0.005	0.001

0	0.001	0	0	0.00001	0.00001	0.006	0.002	0.004
0	0.002	0	0	0.001	0.00001	0.009	0.001	0.004
0.00001	0.00001	0	0.00001	0.00001	0.006	0.00001	0.004	0.00001
0.001	0.002	0.001	0	0.002	0.001	0.018	0.004	0.012
0	0.002	0.00001	0	0.001	0.00001	0.011	0.003	0.007
0	0.002	0.00001	0	0.002	0.001	0.013	0.004	0.01
0	0.002	0.0001	0	0.002	0.001	0.014	0.003	0.008
0	0.002	0.001	0	0.002	0.001	0.017	0.004	0.011
0.001	0.002	0.001	0	0.002	0.001	0.018	0.004	0.012
0	0.002	0.001	0	0.002	0.001	0.019	0.004	0.011
0	0.002	0.001	0	0.002	0.001	0.015	0.004	0.01
0	0.002	0.001	0	0.002	0.001	0.018	0.004	0.011
0.004	0.01	0.004	0.00001	0.007	0.008	0.086	0.015	0.045
0.002	0.004	0.004	0.00001	0.002	0.002	0.03	0.007	0.019
0.002	0.005	0.002	0.002	0.004	0.004	0.038	0.009	0.025
0.002	0.006	0.003	0.075	0.005	0.007	0.059	0.011	0.03
0.004	0.008	0.004	0.001	0.007	0.006	0.061	0.011	0.035
0.006	0.01	0.01	0.002	0.008	0.008	0.082	0.016	0.049
0.003	0.005	0.005	0.001	0.005	0.004	0.045	0.016	0.03
0.187	0.117	0.041	0.054	0.092	0.087	1.358	0.363	1.152
0.212	0.161	0.052	0.042	0.102	0.092	1.504	0.373	1.249
0.108	0.094	0.036	0.037	0.071	0.063	1.024	0.236	0.809
0.119	0.09	0.035	0.038	0.074	0.065	0.993	0.278	0.878
0.088	0.108	0.037	0.027	0.073	0.067	1.009	0.223	0.731
0.258	0.207	0.068	0.052	0.137	0.124	1.984	0.406	1.377
0.117	0.088	0.032	0.043	0.06	0.065	0.775	0.156	0.554
0	0	0	0	0	0.002	0	0.001	0
0	0	0	0	0	0.002	0	0.001	0
0	0	0	0	0	0.001	0	0	0
0	0	0	0	0	0.002	0	0.001	0
0	0	0	0	0	0.002	0	0.002	0
0	0	0	0	0	0.002	0	0.002	0
0	0	0	0	0	0.002	0	0.002	0
0	0	0	0	0	0.004	0.001	0.002	0
0	0	0	0	0	0.001	0	0	0
0	0	0	0	0	0.002	0	0.001	0
0	0	0	0	0	0.001	0	0	0
0.002	0.008	0.003	0.001	0.004	0.006	0.057	0.006	0.018
0.001	0.005	0.002	0.001	0.003	0.004	0.036	0.004	0.012
0.001	0.006	0.002	0.001	0.003	0.005	0.044	0.006	0.017
0.008	0.019	0.007	0.003	0.009	0.012	0.117	0.013	0.041
0.004	0.011	0.004	0.001	0.005	0.007	0.067	0.008	0.023
0.003	0.01	0.005	0.001	0.006	0.007	0.076	0.009	0.027
0.003	0.011	0.005	0.001	0.006	0.009	0.077	0.011	0.032
0.001	0.009	0.004	0.001	0.004	0.006	0.056	0.004	0.013
0.002	0.013	0.009	0.001	0.009	0.013	0.126	0.015	0.056

0.002	0.014	0.006	0.001	0.007	0.011	0.084	0.007	0.024
0.002	0.003	0.001	0	0.003	0.002	0.025	0.003	0.009
0.00001	0.003	0.001	0	0.002	0.002	0.021	0.003	0.011
0.001	0.003	0.001	0	0.003	0.002	0.026	0.004	0.014
0	0.002	0.00001	0	0.002	0.002	0.022	0.003	0.009
0.002	0.004	0.001	0	0.002	0.002	0.023	0.003	0.015
0.0001	0.003	0.001	0	0.001	0.027	0.026	0.013	0.011
0.001	0.004	0.001	0	0.003	0.002	0.027	0.005	0.015
0.002	0.003	0.001	0	0.01	0.027	0.021	0.003	0.011
0.001	0.003	0.001	0	0.03	0.002	0.024	0.004	0.011
0.001	0.003	0.001	0	0.003	0.002	0.026	0.004	0.015
0.03	0.206	0.085	0.007	0.09	0.138	1.451	0.103	0.32
0.027	0.066	0.026	0.009	0.038	0.056	0.462	0.08	0.218
0.027	0.082	0.035	0.007	0.047	0.056	0.563	0.083	0.27
0.018	0.086	0.036	0.006	0.042	0.071	0.596	0.064	0.187
0.014	0.038	0.022	0.004	0.023	0.035	0.27	0.044	0.124
0.034	0.12	0.055	0.01	0.075	0.089	0.875	0.117	0.34
0.026	0.086	0.039	0.009	0.05	0.059	0.61	0.09	0.255
0.03	0.098	0.043	0.008	0.092	0.077	0.676	0.078	0.225
0.044	0.158	0.065	0.012	0.085	0.114	1.173	0.149	0.426

183	183	128	174	177	202	171	156	200
0.003	0.004	0.002	0.003	0.00001	0.003	0.003	0.00001	0.00001
0.002	0.003	0.001	0.00001	0.00001	0.0011	0.002	0.00001	0.00001
0.002	0.003	0.002	0.002	0.00001	0.00001	0.002	0	0.00001
0.002	0.003	0.002	0.002	0.00001	0.001	0.002	0.00001	0.00001
0.003	0.003	0.003	0.003	0.00001	0.001	0.002	0.00001	0.001
0.002	0.005	0.002	0.003	0.00001	0.00001	0.004	0.00001	0.001
0.001	0.003	0.002	0.002	0.001	0	0.002	0.00001	0.00001
0.002	0.003	0.002	0.003	0.001	0.001	0.002	0	0.00001
0.002	0.003	0.002	0.002	0.00001	0.00001	0.002	0.00001	0.00001
0.002	0.002	0.002	0.003	0.00001	0.00001	0.001	0.00001	0.003
0.002	0.003	0.002	0.003	0.00001	0.001	0.002	0.00001	0.001
0.008	0.014	0.006	0.01	0.003	0.003	0.015	0.001	0.004
0.012	0.023	0.009	0.013	0.004	0.004	0.023	0.003	0.006
0.021	0.021	0.021	0.027	0.011	0.007	0.021	0.005	0.006
0.011	0.005	0.008	0.017	0.007	0.003	0.011	0.003	0.003
0.066	0.02	0.016	0.01	0.02	0.011	0.006	0.016	0.006
0.008	0.01	0.008	0.011	0.004	0.003	0.01	0.002	0.003
0.005	0.004	0.005	0.008	0.003	0.002	0.004	0.001	0.002
0.005	0.005	0.005	0.008	0.003	0.002	0.006	0	0.002
0.005	0.003	0.005	0.007	0.003	0.002	0.004	0.001	0.002
0.074	0.034	0.048	0.06	0.017	0.026	0.04	0.01	0.007
0.081	0.112	0.095	0.077	0.016	0.032	0.105	0.01	0.022
0.075	0.061	0.069	0.068	0.017	0.027	0.064	0.011	0.013
0.105	0.052	0.067	0.081	0.02	0.033	0.059	0.013	0.012
0	0	0	0	0	0.001	0	0	0
0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0
0.011	0.009	0.004	0.01	0.007	0.004	0.011	0.003	0.006
0.004	0.003	0.004	0.002	0.003	0.00001	0.00001	0.002	0.013
0.021	0.006	0.005	0.004	0.007	0.004	0.002	0.007	0.002
0.02	0.011	0.007	0.022	0.001	0.007	0.02	0.006	0.006
0.003	0.004	0.003	0.003	0.001	0.001	0.003	0.00001	0.00001
0.012	0.003	0.004	0.003	0.005	0.002	0	0.003	0.001
0.003	0.004	0.002	0.003	0.00001	0.001	0.003	0.0001	0.001
0.003	0.002	0.002	0.00001	0.00001	0.002	0.0001	0.00001	0.001
0.125	0.075	0.107	0.033	0.031	0.118	0.02	0.031	0.041
0.142	0.036	0.074	0.036	0.047	0.014	0.015	0.07	0.009
0.088	0.141	0.078	0.148	0.045	0.041	0.122	0.026	0.032
0.024	0.036	0.024	0.036	0.013	0.01	0.041	0.006	0.011
0.074	0.022	0.03	0.143	0.014	0.025	0.042	0.381	0.218
0.004	0.002	0	0.003	0.003	0.001	0.004	0.002	0
0.003	0.00001	0.00001	0.00001	0.00001	0.00001	0.00001	0.00001	0.00001
0.002	0.001	0.001	0.00001	0.00001	0.002	0	0	0.00001
0.002	0.001	0	0.00001	0.00001	0.002	0.00001	0	0.001

0.002	0.00001	0	0.001	0.00001	0	0.001	0	0.00001
0.001	0.002	0.001	0.002	0.00001	0.00001	0.003	0	0.00001
0.002	0.00001	0	0.00001	0.00001	0.00001	0	0.00001	0.00001

0.003	0.004	0.003	0.003	0.002	0.001	0.003	0.00001	0.001
0.002	0.003	0.002	0.002	0.00001	0.001	0.002	0.00001	0.00001
0.002	0.004	0.003	0.003	0.002	0.001	0.002	0.00001	0.001
0.002	0.004	0.002	0.002	0.002	0.001	0.002	0.00001	0.00001
0.003	0.004	0.002	0.003	0.002	0.001	0.004	0.00001	0.00001
0.003	0.004	0.003	0.003	0.001	0.001	0.003	0.00001	0.001
0.003	0.004	0.002	0.003	0.001	0.001	0.003	0.00001	0.001
0.002	0.004	0.002	0.003	0.001	0.001	0.003	0.00001	0.001
0.003	0.004	0.002	0.003	0.001	0.001	0.002	0.00001	0.001

0.016	0.015	0.011	0.017	0.004	0.006	0.014	0.002	0.004
0.006	0.006	0.006	0.007	0.002	0.002	0.005	0.002	0.002
0.007	0.007	0.006	0.009	0.004	0.002	0.007	0.002	0.002
0.01	0.012	0.006	0.01	0.004	0.003	0.011	0.002	0.003
0.009	0.012	0.009	0.013	0.005	0.004	0.011	0.002	0.003
0.013	0.015	0.011	0.018	0.006	0.005	0.013	0.003	0.004
0.008	0.011	0.007	0.011	0.005	0.003	0.008	0.002	0.003

0.317	0.108	0.176	0.361	0.078	0.112	0.119	0.049	0.042
0.369	0.092	0.229	0.373	0.066	0.081	0.144	0.052	0.044
0.226	0.049	0.185	0.25	0.056	0.071	0.102	0.033	0.029
0.246	0.081	0.153	0.285	0.056	0.081	0.098	0.042	0.03

0.281	0.081	0.141	0.259	0.052	0.059	0.105	0.031	0.031
0.47	0.182	0.268	0.483	0.123	0.156	0.191	0.056	0.059
0.175	0.049	0.127	0.193	0.044	0.09	0.091	0.028	0.027

0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0

0.007	0.01	0.006	0.007	0.02	0.003	0.011	0.001	0.002
0.005	0.01	0.004	0.005	0.001	0.002	0.008	0.00001	0.002
0.006	0.017	0.006	0.007	0.002	0.003	0.009	0.001	0.003
0.015	0.024	0.008	0.017	0.006	0.007	0.023	0.004	0.006
0.009	0.014	0.008	0.01	0.002	0.004	0.011	0.001	0.002
0.01	0.023	0.005	0.008	0.003	0.04	0.015	0.00001	0.004
0.01	0.015	0.01	0.012	0.004	0.005	0.014	0.002	0.004
0.006	0.011	0.005	0.006	0.001	0.003	0.01	0.00001	0.002
0.015	0.012	0.009	0.016	0.006	0.007	0.028	0.003	0.007

0.01	0.018	0.01	0.009	0.002	0.004	0.015	0.001	0.005
0.003	0.005	0.003	0.003	0.002	0.001	0.004	0.00001	0.00001
0.002	0.005	0.002	0.004	0.002	0.001	0.003	0.00001	0.001
0.004	0.006	0.003	0.005	0.002	0.002	0.004	0.00001	0.002
0.003	0.003	0.003	0.003	0.002	0.001	0.003	0.00001	0.001
0.003	0.004	0.002	0.004	0.001	0.001	0.004	0.00001	0.001
0.003	0.005	0.003	0.004	0.001	0.001	0.004	0.00001	0.001
0.003	0.006	0.003	0.004	0.002	0.001	0.006	0.00001	0.002
0.003	0.005	0.003	0.003	0.001	0.001	0.003	0.001	0.001
0.003	0.005	0.003	0.004	0.002	0.001	0.004	0.00001	0.001
0.003	0.05	0.003	0.005	0.002	0.002	0.005	0.00001	0
0.112	0.23	0.086	0.106	0.025	0.043	0.243	0.013	0.065
0.053	0.083	0.053	0.072	0.025	0.024	0.082	0.014	0.024
0.058	0.097	0.059	0.078	0.022	0.027	0.091	0.014	0.037
0.058	0.106	0.047	0.053	0.018	0.022	0.113	0.007	0.031
0.033	0.055	0.032	0.043	0.016	0.014	0.045	0.006	0.013
0.091	0.158	0.088	0.129	0.035	0.047	0.138	0.021	0.045
0.064	0.108	0.061	0.085	0.029	0.03	0.102	0.013	0.035
0.07	0.109	0.063	0.07	0.026	0.028	0.114	0.014	0.033
0.121	0.191	0.103	0.134	0.041	0.049	0.195	0.023	0.066

157	172	180	170-190	201	196-203	208	195	207
0.001	0.01	0.006	0.002	0.003	0.005	0	0	0
0	0.008	0.004	0.002	0.003	0	0.00001	0	0
0.001	0.008	0.005	0.003	0.001	0.00001	0.00001	0	0.001
0.001	0.011	0.005	0.003	0.003	0.002	0.00001	0	0.001
0.001	0.013	0.006	0.002	0.003	0	0.001	0	0
0.002	0.014	0.007	0.004	0.003	0.002	0.001	0	0
0	0.008	0.004	0.002	0.002	0	0.00001	0	0
0.002	0.01	0.006	0.004	0.003	0	0.00001	0	0
0.001	0.008	0.005	0.003	0.003	0.001	0.00001	0	0
0.001	0.009	0.005	0.003	0.003	0	0.00001	0	0
0.002	0.012	0.005	0.003	0.003	0	0.00001	0	0
0.002	0.04	0.023	0.009	0.01	0.002	0.004	0	0.012
0.008	0.068	0.037	0.015	0.015	0.006	0.004	0	0.02
0.012	0.085	0.047	0.034	0.027	0.015	0.008	0.006	0.029
0.008	0.043	0.026	0.02	0.012	0.009	0.004	0.003	0.015
0.005	0.01	0.084	0.039	0.028	0.028	0.028	0.007	0.015
0.006	0.034	0.021	0.012	0.01	0.004	0.004	0	0.012
0.003	0.021	0.012	0.009	0.008	0.004	0.002	0	0.007
0.004	0.02	0.014	0.009	0.006	0.004	0.002	0	0.007
0.003	0.021	0.011	0.008	0.006	0.002	0.002	0	0.007
0.032	0.329	0.148	0.065	0.082	0.013	0.023	0.009	0.096
0.034	0.356	0.191	0.062	0.067	0.01	0.023	0	0.007
0.033	0.332	0.164	0.064	0.073	0.012	0.025	0	0.008
0.045	0.485	0.219	0.082	0.11	0.015	0.034	0.011	0.143
0.005	0.002	0	0	0	0	0	0	0
0.002	0	0	0	0	0	0	0	0
0.003	0.001	0	0	0	0	0	0	0
0.045	0.021	0.018	0.014	0.023	0.003	0.003	0.008	0.011
0.007	0.005	0.004	0.003	0.005	0.004	0.003	0.00001	0
0.002	0.004	0.023	0.013	0.01	0.007	0.01	0.002	0.004
0.013	0.109	0.051	0.035	0.035	0.019	0.009	0.014	0.032
0.002	0.012	0.006	0.004	0.003	0.003	0.00001	0	0.003
0.00001	0.002	0.014	0.008	0.005	0.004	0.004	0.001	0
0.002	0.011	0.006	0.003	0.003	0.002	0.00001	0	0.003
0.007	0.004	0.002	0.002	0.002	0.002	0.00001	0.00001	0
0.379	0.207	0.102	0.101	0.04	0.031	0.021	0.095	0.037
0.018	0.022	0.178	0.103	0.051	0.047	0.017	0.016	0.011
0.05	0.389	0.233	0.162	0.145	0.054	0.052	0.037	0.04
0.016	0.114	0.071	0.042	0.036	0.014	0.012	0.008	0.045
0.087	0.042	0.381	0.218	0.087	0.084	0.026	0.029	0.012
0.003	0.021	0.008	0.009	0.011	0.002	0.006	0.006	0.006
0	0.004	0.002	0.002	0.002	0.001	0.003	0	0
0.005	0.003	0.002	0.001	0.001	0.00001	0	0	0
0.006	0.004	0.003	0.002	0.001	0.00001	0	0	0

0	0.004	0.003	0.002	0.002	0	0	0	0.001
0.00001	0.006	0.002	0.002	0.002	0.002	0.00001	0	0.001
0.005	0.003	0.001	0.001	0	0.00001	0	0	0
0.002	0.011	0.007	0.004	0.004	0.002	0.00001	0.00001	0.003
0.001	0.008	0.004	0.003	0.003	0.001	0.00001	0.00001	0.002
0.002	0.011	0.006	0.004	0.003	0	0.00001	0.001	0.003
0.001	0.009	0.005	0.004	0.003	0.002	0.00001	0.00001	0.002
0.001	0.012	0.007	0.005	0.003	0.003	0.00001	0.00001	0.003
0.002	0.013	0.008	0.005	0.004	0.003	0.00001	0.001	0.004
0.001	0.011	0.007	0.004	0.003	0.002	0.00001	0.00001	0.003
0.001	0.012	0.006	0.004	0.003	0.002	0.00001	0.00001	0.003
0.002	0.01	0.007	0.004	0.003	0.002	0.00001	0.00001	0.003
0.007	0.06	0.035	0.015	0.015	0.004	0.006	0.002	0.022
0.002	0.024	0.013	0.008	0.006	0.004	0.002	0.0001	0.006
0.004	0.03	0.017	0.01	0.009	0.004	0.003	0.002	0.011
0.005	0.037	0.021	0.011	0.011	0.004	0.003	0.0001	0.012
0.006	0.033	0.021	0.014	0.012	0.005	0.003	0.001	0.013
0.007	0.05	0.028	0.017	0.014	0.006	0.005	0.002	0.015
0.005	0.03	0.017	0.013	0.009	0.004	0.003	0.002	0.01
0.124	1.206	0.564	0.266	0.352	0.053	0.1	0.04	0.375
0.139	1.441	0.663	0.297	0.4	0.046	0.116	0.041	0.442
0.096	0.977	0.409	0.196	0.248	0.034	0.068	0.026	0.293
0.099	0.893	0.429	0.216	0.285	0.035	0.087	0.032	0.33
0.092	0.929	0.434	0.175	0.271	0.03	0.081	0.031	0.321
0.162	1.802	0.815	0.319	0.491	0.053	0.143	0.052	0.556
0.073	0.728	0.336	0.147	0.202	0.025	0.06	0.026	0.248
0.001	0	0	0	0	0	0	0	0
0.001	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0
0.002	0.001	0	0	0	0	0	0	0
0.002	0	0	0	0	0	0	0	0
0.002	0.001	0	0	0	0	0	0	0
0.002	0.001	0.001	0	0	0	0	0	0
0.003	0.001	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0
0.001	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0
0.003	0.029	0.018	0.007	0.008	0.002	0.002	0	0.008
0.003	0.02	0.011	0.006	0.005	0	0.002	0	0.006
0.005	0.025	0.017	0.006	0.008	0.003	0.003	0.001	0.009
0.005	0.063	0.039	0.019	0.021	0.01	0.007	0.005	0.022
0.003	0.029	0.019	0.008	0.009	0	0.002	0	0.01
0.006	0.038	0.023	0.009	0.01	0.005	0.007	0.002	0.01
0.006	0.044	0.024	0.012	0.012	0.003	0.004	0.002	0.014
0.003	0.022	0.014	0.006	0.006	0.001	0.002	0	0.004
0.009	0.08	0.045	0.02	0.022	0.008	0.007	0.003	0.024

0.005	0.031	0.022	0.01	0.008	0.002	0.003	0	0.008
0.003	0.017	0.009	0.006	0.004	0.004	0.00001	0.0001	0.004
0.002	0.012	0.007	0.005	0.003	0.005	0.00001	0.001	0.003
0.003	0.017	0.01	0.007	0.005	0.004	0.002	0.001	0.005
0.001	0.011	0.006	0.005	0.004	0.003	0.001	0.002	0.003
0.002	0.013	0.008	0.005	0.004	0.003	0.001	0.00001	0.004
0.002	0.014	0.008	0.004	0.004	0.002	0.001	0.001	0.003
0.003	0.014	0.009	0.007	0.004	0.005	0.002	0.001	0.005
0.002	0.013	0.007	0.004	0.003	0.002	0.00001	0.001	0.004
0.003	0.014	0.008	0.006	0.004	0.004	0.001	0.002	0.004
0.003	0.016	0.009	0.007	0.004	0.004	0.001	0.001	0.005
0.066	0.682	0.345	0.103	0.14	0.029	0.04	0.01	0.184
0.029	0.221	0.131	0.082	0.069	0.026	0.025	0.013	0.07
0.032	0.257	0.144	0.079	0.074	0.03	0.026	0.012	0.078
0.035	0.298	0.156	0.065	0.072	0.022	0.017	0.014	0.083
0.018	0.15	0.08	0.047	0.044	0.015	0.015	0.032	0.094
0.046	0.381	0.217	0.133	0.116	0.048	0.044	0.025	0.12
0.037	0.305	0.177	0.094	0.093	0.035	0.029	0.013	0.04
0.038	0.335	0.178	0.075	0.086	0.023	0.035	0.006	0.08
0.066	0.61	0.33	0.161	0.161	0.051	0.054	0.032	0.103

194	206	%LIP	A1254_60	A1260	MEANRL	MEANRPL	MEANRK
0.003	0.001	10.39	0.19	0.07	6.2	146	1
0.003	0.001	8.72	0.192	0.091			
0.002	0.00001	9.02	0.22	0.12			
0.003	0.001	7.74	0.15	0.07			
0.002	0.001	9.12	0.16	0.09			
0.003	0.002	11.24	0.19	0.07			
0.002	0	8.89	0.19	0.08			
0.002	0	8.5	0.22	0.11			
0.003	0.00001						
0.002	0.001						
0.003	0.001						
0.003	0.003	10.13	1	0.36	6.1	83	0.6
0.006	0.006	16.64	1.5	0.62			
0.012	0.012	12.99	2	0.9			
0.007	0.007	8.87	1.53	0.77			
0.021	0.016	19.67	0.7	0.4			
0.005	0.005	7.01	0.64	0.31			
0.03	0.03	12.38	1.13	0.76			
0.003	0.003	15.86	0.38	0.19			
0.003	0.003	13.28	0.34	0.18			
0.02	0.02	9.77	4.8	3			
0.077	0.015	13.9	10	3.2	2.7	65	0.6
0.087	0.016	13.95	6.7	3			
0.025	0.025	5.97	6.6	4.4			
0.001	0	7.32	0.08	0.04	5.8	121	0.7
0	0	7.6	0.04	0.02			
0	0	10.25	0.04	0.03			
0.013	0.013	12.03	0.78	0.41	5	74	0.61
0.002	0.0002	13.08	0.3	0.14			
0.007	0.007	8.19	0.425	0.21			
0.014	0.014	12.9	1.1	0.99			
0.002		7.18	0.23	0.09			
0.004	0.003	9.94	0.247	0.13			
0.003	0.001	9.27	0.24	0.1			
0.002	0.0001	8.99	0.2	0.1			
0.06	0.043	6.05	5.5	1.6	7.2	120	0.76
0.056	0.02	13.75	11.6	3.5			
0.145	0.067	12.44	3	1			
0.018	0.018	10.97	10.3	3.4			
0.107	0.031	15.53	0.2	0.19			
0.006	0.006	9.21	11.9	3.4			
0	0	10.35	0.08	0.03			
0.002	0.001		0.068	0.036			
0.002	0.00001	7.24	0.1	0.05			

0.001	0.001	7.91	0.12	0.05			
0.001	0.001	11.41					
0	0.00001	9.24	0.12	0.05			
0.003	0.002	6.82	0.24	0.09	5.2	63	1.02
0.002	0.00001	12.44	0.24	0.1			
0.003	0.002	7.77	0.15	0.07			
0.002	0.001	10.33	0.18	0.09			
0.003	0.002	8.65	0.19	0.08			
0.004	0.001	11.43	0.23	0.11			
0.003	0.002	10.67	0.24	0.12			
0.003	0.001	10.52	0.26	0.1			
0.003	0.002	9.53	0.2	0.1			
0.022	0.005	9.04	1.2	0.55	4.6	42	0.88
0.003	0.003	6.35	0.41	0.22			
0.011	0.004	6.73	0.52	0.27			
0.012	0.004	13.27	0.81	0.338			
0.003	0.003	9	0.83	0.3			
0.007	0.007	13.86	1.121	0.46			
0.004	0.004	12.05	0.62	0.27			
0.375	0.069	10.45	18.6	11	7.5	67	1.3
0.442	0.086	14.34	20.6	13			
0.293	0.053	5.41	14.1	8.9			
0.33	0.066	6.33	13.6	8.2			
0.321	0.061	10.99	27.2	16.4	13.5	93	1.4
0.556	0.101	6.94	13.8	8.47			
0.248	0.049	7.4	10.6	6.6			
0	0	9.83	0.03	0.009	9.4	88	1.4
0	0	11.51	0.02	0.009			
0	0	5.46	0.05	0.027			
0	0	1.22	0.027	0.018			
0	0	7.11	0.03	0.02			
0	0	8.18	0.027	0.018			
0	0	14.16	0.027	0.018			
0	0	11.85	0.014	0.008			
0	0	10.7	0.014	0.009			
0	0	3.43	0.014	0.009			
0	0	5.92	0.014	0.009			
0.002	0.003	12.36	0.98	0.3	9.7	118	1.5
0.001	0.005	10.88	0.78	0.26			
0.003	0.008	8.29	0.49	0.18			
0.01	0.003	3.99	0.6	0.22			
0.003	0.004	3.87	1.6	0.58			
0.01	0.002	12.72	0.92	0.265			
0.006	0.005	9.92	1.04	0.347			
0.001	0.008	11.87	1.05	0.401			
0.008	0.006	10.94	0.767	0.201			

0.002	0.014	10.02	1.7	0.73			
0.003	0.003	8.48	0.34	0.16	8.6	92	1.15
0.002	0.002	7.77	0.29	0.109			
0.003	0.003	11.61	0.41	0.21			
0.003	0.002	5.11	0.3	0.1			
0.002	0.002	11.92	0.315	0.119			
0.002	0.002	9.66	0.356	0.128			
0.005	0.003	9.22	0.37	0.12			
0.002	0.002	14.36	0.288	0.119			
0.003	0.003	5.23	0.329	0.128			
0.003	0.003	10.32	0.356	0.146			
0.04	0.034	11.13	19.8	6.22	7.9	69	1.15
0.026	0.025	10.2	6.3	2			
0.03	0.025	11.25	7.7	2.3			
0.017	0.023		8.16	2.7			
0.015	0.015	6.93	3.6				
0.048	0.042	14.44	16	4.5			
0.029	0.027	10.71	11.9	3.4			
0.035	0.023	9.34	8.3	2.78			
0.051	0.054	14.08	9.2	3.1			

MEANRPK	MEANRAT	MEANRAT	EGGPCB	STATUS	SUMTEQ	MR2K	MR2L	MPCB
5.2	0.08	0.42	0.771	c	51	1.338887	0.025251	0.205
				c				
				c				
				c				
				c				
				c				
				c				
				c				
2.8	0.14	0.51	2.104	c	332	0.797988	0.035723	1.9
				c				
				c				
				c				
				c				
				c				
				c				
				c				
				c				
				d				
1.6	0.2	0.77		d	2851	1.628181	0.044102	31.75
				d				
				d				
2.3	0.09	0.58		c	14	0.898482	0.048756	0.025
				c				
				c				
2.6	0.12	0.5	0.654	c	86	1.075101	0.042123	0.36
				c				
				c				
				c				
				c				
				c				
				c				
				c				
2.6	0.11	0.49	41.824	d	3998	0.518131	0.081338	29.886
				d				
				d				
				d				
				d				
				d				
	0.1	0.6	0.209	d	43	1.65933	0.04224	0.09
				d				
				d				

			d				
			d				
			d				
1.9	0.16	1.2	c	68	2.505874	0.056552	0.225
			c		4.198082	0.049687	0.225
			c				
			c				
			c				
			c				
			c				
			c				
			c				
2.12	0.18	1.1	d	352	1.302684	0.033396	1.5
			d		2.214128	0.06272	1.6
			d				
			d				
			d				
			d				
			d				
1.1	0.19	3.2	d	2979	6.34836	0.069236	58.6
			d				
			d				
			d				
2.2	0.29	3.4	d		9.544642	0.114385	69
			d				
			d				
3	0.2	1.1	c	11	2.65886	0.094487	0.013
			c		1.412866	0.087505	0.013
			c				
			c				
			c				
			c				
			c				
			c				
			c				
			c				
			c				
5.9	0.165	0.8	d	715	1.520221	0.073425	2.1
			d		1.784618	0.056552	2.1
			d		0.900455	0.075985	2.1
			d				
			d				
			d				
			d				
			d				
			d				

			d				
3.2	0.16	0.9	d	124	3.328323	0.043403	0.31
			d		1.955514	0.134748	0.31
			d				
			d				
			d				
			d				
			d				
			d				
			d				
			d				
2.4	0.19	1.6	d	6487	2.099388	0.086574	51.59
			d		1.870804	0.082618	51.95
			d				
			d				
			d				
			d				
			d				
			d				
			d				

PCB118	LPCB118	JULIANDA	YR	EROD	EROD2	INSVITA	BCAROTE	RETLIV2
0.013	-1.88606	174	1999	11	26.39	0.08	2.75	5.03
0.016	-1.79588	167	1999	24.7				
0.013	-1.88606	176	1999	50.1				
0.008	-2.09691	167	1999	18				
0.009	-2.04576	167	1999	15				
0.011	-1.95861	166	1999	25				
0.01	-2	170	1999	26.3				
0.008	-2.09691	167	1999	41				
0.173	-0.76195	172	1999	33.8	41.53	0.12	2.13	4.7
0.257	-0.59007	165	1999	44.1				
0.038	-1.42022	168	1999	41.5				
0.205	-0.68825	166	1999	12.1				
0.041	-1.38722	161	1999	59				
0.062	-1.20761	165	1999	29.1				
0.051	-1.29243	166	1999	25.4				
0.021	-1.67778	163	1999	73.4				
0.029	-1.5376	163	1999	53.2				
0.4	-0.39794	170	1999	169.4				
1.46	0.164353	188	1999	48.5				
0.715	-0.14569	182	1999		60.7	0.15	6.34	5.77
0.002	-2.69897	163	1999	72.9				
0.003	-2.52288	170	1999	7.7	17.37	0.15	1.51	5.83
0.002	-2.69897	169	1999	24.5				
0.002	-2.69897	170	1999	19.9				
0.036	-1.4437	162	1999	17.8	57.03	0.07	8.72	3
0.015	-1.82391	159	1999	68				
0.02	-1.69897	171	1999	91.6				
0.043	-1.36653	160	1999	29.9				
0.013	-1.88606	167	1999	15.3				
0.011	-1.95861	174	1999	134.9				
		174	1999	60.7				
0.013	-1.88606	172	1999	38				
1.207	0.081707	172	1999	45.2	57.82	0.24	3.49	7.76
1.918	0.282849	155	1999	67.6				
0.566	-0.24718	161	1999	57.3				
0.249	-0.6038	172	1999	51.2				
0.009	-2.04576	159	1999	54.1				
2.049	0.311542	163	1999	71.5				
0.003	-2.52288	170	1999	27.6				4.37
		172	1999	22				
0.004	-2.39794	168	1999	26				

0.007	-2.1549	171	1999	35.8			
		166	1999	12.8			
0.005	-2.30103	169	1999	13.1			
		164	2000		0.08	2.75	7.1
0.014	-1.85387	170	2000				5.9
0.008	-2.09691	170	2000				
0.009	-2.04576	175	2000				
0.011	-1.95861	168	2000				
0.012	-1.92082	172	2000				
0.013	-1.88606	171	2000				
0.014	-1.85387	168	2000				
0.011	-1.95861	172	2000				
0.128	-0.89279	195	2000		0.12	2.13	3.6
0.03	-1.52288	181	2000				6.3
0.04	-1.39794	188	2000				
0.106	-0.97469	188	2000				
0.09	-1.04576	164	2000				
0.109	-0.96257	170	2000				
0.041	-1.38722	168	2000				
0.732	-0.13549	164	2000		0.12	8.11	8.9
0.854	-0.06854	174	2000				
0.643	-0.19179	188	2000				
0.0557	-1.25414	176	2000				
1.39	0.143015	169	2000		0.15	6.34	13.5
0.726	-0.13906	174	2000				
0.624	-0.20482	178	2000				
0.001	-3	172	2000		0.15	1.51	11.26
0.001	-3	172	2000				9.8
0.002	-2.69897	171	2000				
0.001	-3	165	2000				
0.002	-2.69897	169	2000				
0.002	-2.69897	168	2000				
0.001	-3	172	2000				
0.0001	-4	175	2000				
0.001	-3	172	2000				
0.0001	-4	175	2000				
0.0001	-4	169	2000				
0.076	-1.11919	169	2000		0.12	3	8.3
0.077	-1.11351	176	2000				13.2
0.052	-1.284	179	2000				8.6
0.058	-1.23657	177	2000				
0.302	-0.51999	179	2000				
0.082	-1.08619	173	2000				
0.177	-0.75203	179	2000				
0.087	-1.06048	169	2000				
0.065	-1.18709	172	2000				

0.265	-0.57675	176	2000			
0.017	-1.76955	170	2000	0.07	8.72	4.56
0.014	-1.85387	164	2000			14.5
0.018	-1.74473	167	2000			
0.014	-1.85387	160	2000			
0.016	-1.79588	170	2000			
0.019	-1.72125	181	2000			
0.018	-1.74473	164	2000			
0.016	-1.79588	173	2000			
0.016	-1.79588	168	2000			
0.018	-1.74473	167	2000			
4.698	0.671913	167	2000	0.24	3.49	13.8
1.331	0.124178	160	2000			9.2
1.736	0.23955	159	2000			
1.904	0.279667	171	2000			
0.754	-0.12263	164	2000			
3.492	0.543074	174	2000			
2.629	0.419791	177	2000			
1.73	0.238046	164	2000			
2.231	0.3485	164	2000			

RPLIV2	R_RPL2	RETKI2	RPKID2	R_RPK2	NOPCB	PCB77	CTEQ77	TEQ77
150	1.01	0.9	3.36	0.267	890.1	681	20.43	0.6129
97	1	0.7	3.6	0.19	10007.79	8218	246.54	7.396
56	1.453401	0.6	1.67	0.359281	117137.2	70505	2115.15	63.45
121	1.219665	0.7	2.3	0.304348	297.4	207	6.21	0.1863
87	0.69	0.53	1.8	0.29	1556.1	1212	36.36	1.0908
120	1.6	0.93	6.4	0.14	159640.6	94628	2838.84	85.165
100.2	0.962555	0.67	3.74	0.167	642.9	469	14.07	0.4221

68.6	0.103499	1.3	2.9	0.448276	2483.3	1100	33	0.099
93	0.063441	0.63	1.1	0.572727	2752.2	1300	39	0.117
36.6	0.098361	1.2	2.6	0.461538	11899.7	8100	243	0.729
50.6	0.124506	0.96	1.8	0.533333	15705	8400	252	0.756
80	0.11125	1.4	1.26	1.111111	150199	76000	2280	6.84
93	0.145161	1.4	2.2	0.636364	29596			
115	0.097913	1.7	3.73	0.455764	241.7	110	3.3	0.0099
100.1	0.097902	1.16	3.53	0.328612	382.2	170	5.1	0.0153
130	0.063846	2.26	6.8	0.332353	15356.7	5700	171	0.513
100.1	0.131868	0.6	0.9	0.666667	55515	34000	1020	3.06
125	0.0688	1.53	8.3	0.184337	48440	17000	510	1.53

78	0.058462	1	1.6	0.625	4140	1700	51	0.153
127	0.114173	0.9	2.26	0.39823	4373	2100	63	0.189
77.6	0.177835	0.83	1.5	0.553333	490470	220000	6600	19.8
105	0.087619	2.1	4.7	0.446809	318740	120000	3600	10.8

PCB169	CTEQ169	TEQ169	PCB126	CTEQ126	TEQ126	PCB37	CTEQ37	PCDD
4	0.08	0.012	66	19.8	19.8	113	0.0452	13.75
7	0.14	0.021	238	71.4	71.4	1307.77	0.523108	8.13
37	0.74	0.111	2307	692.1	692.1	41524.7	16.60988	19.31
2	0.04	0.006	19	5.7	5.7	59	0.0236	5.45
8	0.16	0.024	100	30	30	197	0.0788	13.59
38	0.76	0.114	3647	1094.1	1094.1	56183	22.4732	8.21
6	0.12	0.018	53	15.9	15.9	98	0.0392	34.64

5.3	0.106	0.0159	68	20.4	20.4	110	0.044	7.73
6.2	0.124	0.0186	76	22.8	22.8	1100	0.44	6.11
9.7	0.194	0.0291	230	69	69	9900	3.96	10.9
15	0.3	0.045	290	87	87	5900	2.36	11.93
99	1.98	0.297	2100	630	630	47000	18.8	32.43
96			2500					27.86
2.7	0.054	0.0081	12	3.6	3.6	92	0.0368	6.405
3.2	0.064	0.0096	19	5.7	5.7	150	0.06	6.87
6.7	0.134	0.0201	150	45	45	8900	3.56	20.68
15	0.3	0.045	800	240	240	19000	7.6	29.61
10	0.2	0.03	330	99	99	30000	12	24.09

10	0.2	0.03	120	36	36	1900	0.76	22.54
13	0.26	0.039	150	45	45	1600	0.64	18.34
70	1.4	0.21	5400	1620	1620	250000	100	15.91
40	0.8	0.12	2900	870	870	190000	76	14.55

DIO_FUR	CTEQ2378	D2378	TEQ4D	D12378	CTEQ1237	tetraCDD	PentaCDD	HexaCDD
34.77	1.08	1.08	1.08	2.31	2.541	1.08	3.1	5.36
32.54	0.94	0.94	0.94	1.49	1.639	0.94	1.49	3.05
						0.76	2.53	13.68
82.27	0.57	0.57	0.57	1.63	4.53629	0.57	1.63	5.75
10.23	0.37	0.37	0.37	0.57	0.627	0.37	0.57	2
43.88	1.61	1.61	1.61	2.16	2.376	1.61	2.55	6.13
102.32	1.01	1.01	1.01	1.29	1.419	1.01	1.29	2.4
54.01	1.96	1.96	1.96	5.67	6.237	1.96	7.24	14.91

25.44	0.823	0.823	0.823	0.98	1.078	0.833	1.15	3.18
30.11	0.951	0.951	0.951	0.913	1.0043	1.1	1.16	1.66
48.8	1.13	1.13	1.13	1.25	5.670363	1.3	1.55	3.93
61.45	1.7	1.7	1.7	1.52	1.672	1.97	1.77	3.88
153.02	1.28	1.28	1.28	2.55	2.805	1.38	2.75	10.3
134.27	1.21	1.21	1.21	2.03	2.233	1.21	2.17	8.68
11.424	0.258	0.258	0.258	0.623	0.6853	0.258	0.787	2.73
11.988	0.262	0.262	0.262	0.688	0.7568	0.262	0.828	3.06
46.48	0.68	0.68	0.68	1.1	1.21	0.966	1.1	4.32
63.87	0.89	0.89	0.89	1.53	1.683	1.15	1.77	7.89
58.18	0.806	0.806	0.806	1.23	1.353	1.13	1.51	6.25

75.64	1.94	1.94	1.94	2.27	2.497
65.69	2.37	2.37	2.37	2.6	2.86
168.05	1.31	1.31	1.31	1.56	1.716
119.52	1.28	1.28	1.28	1.64	1.804

SeptaCDD	tetraCDF	PentaCDF	HexaCDF	SeptaCDF	TEQ5D	PCDF	F2378	CTEQ2378
4.21	12.25	4.08	3.79	0.9	0.0231	21.02	7.2	7.92
2.65	17.06	5.09	1.84	0.42	0.0149	24.41	10.7	11.77
62.58	15.61	9.96	10.82	3.26				
11.36	38.71	13.59	8.26	2.4	0.0163	62.96	19.6	21.56
2.51	1.99	1.85	0.76	0.18	0.0057	4.78	1.13	1.243
3.3	21.38	5.97	2.39	0.56	0.0216	30.29	14.2	15.62
5.51	62.64	21.64	8.51	1.29	0.0129	94.11	35.9	39.49
10.53	7.46	5.44	5.55	0.92	0.0567	19.37	4.8	5.28

2.57	12.71	3.79	0.939	0.283	0.0098	17.71	7.25	7.975
2.19	17.9	4.95	1.15	0	0.00913	24	9.04	9.944
4.12	27.12	8.62	2.12	0.138	0.0125	37.9	13.3	14.63
4.31	35.8	11.3	2.82	0	0.0152	49.52	20.2	22.22
18	59.2	39.6	20.2	1.59	0.0255	120.59	41	45.1
15.8	43.8	32.2	23.8	6.51	0.0203	106.41	3.33	3.663
2.63	1.84	2.28	0.899	0	0.00623	5.019	0.982	1.0802
2.72	2.08	2.22	0.818	0	0.00688	5.118	1.19	1.309
14.3	13.9	6.48	3.65	1.77	0.011	25.8	7.11	7.821
18.8	17.8	11.4	5.06	0	0.0153	34.26	10.4	11.44
15.2	21.4	7.92	4.4	0.376	0.0123	34.09	10.6	11.66

0.0227	53.1	18.4	20.24
0.026	47.35	20.9	22.99

0.0156	152.14	54.6	60.06
0.0164	104.97	37.3	41.03

TEQF4	PPDDE	LOGDDE	CHC	LOGCHC	PCB	LOGPCB	CTPCBTE	TPCBTE
0.648	0.018	-1.74473	0.029	-1.5376	0.236	-0.62709	6.78E-05	6.6E-06
	0.015	-1.82391	0.025	-1.60206	0.352	-0.45346		
	0.161	-0.79317	0.211	-0.67572	0.193	-0.71444		
	0.014	-1.85387	0.017	-1.76955	0.145	-0.83863		
	0.019	-1.72125	0.026	-1.58503	0.17	-0.76955		
	0.024	-1.61979	0.035	-1.45593	0.187	-0.72816		
	0.023	-1.63827	0.031	-1.50864	0.169	-0.77211		
	0.015	-1.82391	0.021	-1.67778	0.188	-0.72584		
0.963	0.017	-1.76955	0.033	-1.48149	2.48	0.394452	0.000493	5.34E-05
	0.028	-1.55284	0.057	-1.24413	2.998	0.476832		
	0.038	-1.42022	0.07	-1.1549	3.82	0.582063		
	0.059	-1.22915	0.102	-0.9914	6.743	0.828853		
	0.039	-1.40894	0.065	-1.18709	1.059	0.024896		
	0.031	-1.50864	0.057	-1.24413	0.949	-0.02273		
	0.798	-0.098	0.872	-0.05948	1.204	0.080626		
	0.023	-1.63827	0.046	-1.33724	0.481	-0.31785		
	0.024	-1.61979	0.045	-1.34679	0.478	-0.32057		
	0.015	-1.82391	0.02	-1.69897	10.48	1.020361		
1.764	0.011	-1.95861	0.011	-1.95861	50.187	1.700591	0.000758	7E-05
	0.013	-1.88606	0.013	-1.88606	28.6	1.456366		
	0.028	-1.55284	0.038	-1.42022	16.47	1.216694		
0.1017	0.016	-1.79588	0.018	-1.74473	0.042	-1.37675	4.25E-06	2.79E-07
	0.008	-2.09691	0.008	-2.09691	0.012	-1.92082		
	0.017	-1.76955	0.017	-1.76955	0.021	-1.67778		
1.278	0.021	-1.67778	0.039	-1.40894	0.614	-0.21183	9.74E-05	9.94E-06
	0.025	-1.60206	0.042	-1.37675	0.32	-0.49485		
	0.029	-1.5376	0.054	-1.26761	0.455	-0.34199		
	0.061	-1.21467	0.144	-0.84164	0.95	-0.02228		
	0.023	-1.63827	0.036	-1.4437	0.24	-0.61979		
	0.022	-1.65758	0.044	-1.35655	0.091	-1.04096		
	0.022	-1.65758	0.043	-1.36653	0.1	-1		
	0.031	-1.50864	0.049	-1.3098	0.172	-0.76447		
3.231	0.026	-1.58503	0.046	-1.33724	26.871	1.429284	0.004872	0.000533
	0.051	-1.29243	0.126	-0.89963	43.63	1.639785		
	0.022	-1.65758	0.048	-1.31876	13.058	1.115877		
	0.029	-1.5376	0.056	-1.25181	52.1	1.716838		
	0.229	-0.64016	0.333	-0.47756	33.4	1.523746		
	0.023	-1.63827	0.046	-1.33724	10.26	1.011147		
0.432	0.012	-1.92082	0.013	-1.88606	0.048	-1.31876	2.14E-05	2.25E-06
	0.117	-0.93181	0.217	-0.66354				
	0.015	-1.82391	0.015	-1.82391	0.081	-1.09151		

	0.021	-1.67778	0.023	-1.63827	0.1	-1		
	0.014	-1.85387	0.015	-1.82391	0.094	-1.02687		
	0.021	-1.67778	0.025	-1.60206	0.108	-0.96658		
0.6525	0.022	-1.65758	0.035	-1.45593	0.252	-0.5986	6.78E-05	6.06E-06
0.8136	0.019	-1.72125	0.023	-1.63827	0.269	-0.57025		
	0.009	-2.04576	0.019	-1.72125	0.153	-0.81531		
	0.013	-1.88606	0.036	-1.4437	0.183	-0.73755		
	0.019	-1.72125	0.024	-1.61979	0.205	-0.68825		
	0.016	-1.79588	0.021	-1.67778	0.236	-0.62709		
	0.019	-1.72125	0.025	-1.60206	0.244	-0.61261		
	0		0.026	-1.58503	0.266	-0.57512		
	0.014	-1.85387	0.015	-1.82391	0.2	-0.69897		
1.197	0.015	-1.82391	0.025	-1.60206	2.707	0.432488	0.000493	3.12E-05
1.818	0.02	-1.69897	0.09	-1.04576	0.582	-0.23508		
	0.019	-1.72125	0.038	-1.42022	0.912	-0.04001		
	0.021	-1.67778	0.048	-1.31876	1.783	0.251151		
	0.03	-1.52288	0.062	-1.20761	1.977	0.296007		
	0.048	-1.31876	0.089	-1.05061	2.262	0.354493		
	0.034	-1.46852	0.075	-1.12494	0.971	-0.01278		
3.69	0.033	-1.48149	0.041	-1.38722	71.221	1.852608	0.004625	0.000171
	0.05	-1.30103	0.061	-1.21467	60.941	1.78491		
	0.03	-1.52288	0.03	-1.52288	47.99	1.681151		
	0.022	-1.65758	0.024	-1.61979	54.433	1.735862		
0.2997	0.033	-1.48149	0.038	-1.42022	114.3	2.058046	0.000758	0.000219
	0.022	-1.65758	0.025	-1.60206	51.36	1.710625		
	0.014	-1.85387	0.017	-1.76955	41.75	1.620656		
0.08838	0.006	-2.22185	0.011	-1.95861	0.015	-1.82391	4.25E-06	1.35E-07
0.1071	0.008	-2.09691	0.008	-2.09691	0.008	-2.09691		
	0.017	-1.76955	0.021	-1.67778	0.037	-1.4318		
	0.003	-2.52288	0.004	-2.39794	0.01	-2		
	0.014	-1.85387	0.015	-1.82391	0.015	-1.82391		
	0.02	-1.69897	0.021	-1.67778	0.018	-1.74473		
	0.018	-1.74473	0.027	-1.56864	0.019	-1.72125		
	0.017	-1.76955	0.021	-1.67778	0.004	-2.39794		
	0.014	-1.85387	0.016	-1.79588	0.005	-2.30103		
					0.008	-2.09691		
	0.006	-2.22185	0.006	-2.22185	0.004	-2.39794		
0.6399	0.017	-1.76955	0.023	-1.63827	1.27	0.103804	2.14E-05	5.29E-05
0.936	0.016	-1.79588	0.021	-1.67778	1.475	0.168792		
0.954	0.018	-1.74473	0.021	-1.67778	1.106	0.043755		
	0.014	-1.85387	0.016	-1.79588	0.766	-0.11577		
	0.013	-1.88606	0.013	-1.88606	5.456	0.736874		
	0.021	-1.67778	0.026	-1.58503	1.817	0.259355		
	0.014	-1.85387	0.017	-1.76955	3.456	0.538574		
	0.016	-1.79588	0.021	-1.67778	1.684	0.226342		
	0.01	-2	0.01	-2	1.149	0.06032		

	0.023	-1.63827	0.025	-1.60206	2.989	0.475526		
1.656	0.036	-1.4437	0.057	-1.24413	0.3	-0.52288	9.74E-05	7.15E-06
1.881	0.024	-1.61979	0.043	-1.36653	0.251	-0.60033		
	0.032	-1.49485	0.049	-1.3098	0.335	-0.47496		
	0.024	-1.61979	0.037	-1.4318	0.239	-0.6216		
	0.038	-1.42022	0.06	-1.22185	0.352	-0.45346		
	0.026	-1.58503	0.041	-1.38722	0.345	-0.46218		
	0.034	-1.46852	0.053	-1.27572	0.345	-0.46218		
	0.027	-1.56864	0.049	-1.3098	0.32	-0.49485		
	0.03	-1.52288	0.047	-1.3279	0.305	-0.5157		
	0.038	-1.42022	0.063	-1.20066	0.324	-0.48945		
4.914	0.024	-1.61979	0.039	-1.40894	75.05	1.875351	0.004872	0.001011
3.357	0.025	-1.60206	0.04	-1.39794	35.357	1.548475		
	0.029	-1.5376	0.054	-1.26761	39.288	1.59426		
	0.029	-1.5376	0.042	-1.37675	32.46	1.511349		
	0.03	-1.52288	0.053	-1.27572	14.665	1.166282		
	0.041	-1.38722	0.062	-1.20761	91.832	1.962994		
	0.03	-1.52288	0.049	-1.3098	70.998	1.851246		
	0.034	-1.46852	0.054	-1.26761	36.935	1.567438		
	0.029	-1.5376	0.057	-1.24413	51.26	1.709779		

RETLIVER	LOGRL	RETPAL	LOGRPL	R_RPLIV	RETKID	LOGRK	RETPALK	LOGRPK
1.1	0.041393	30	0.531632	0.323416	0.8	-0.09691	3.2	0.50515
5	0.69897	161	0.705984	0.98398	1.3	0.113943	8.3	0.919078
9.9	0.995635	203	0.725357	1.863282	1.5	0.176091	10.2	1.0086
9.4	0.973128	184	0.717249	1.802515				
3.5	0.544068	132	0.688669	0.716802	1.1	0.041393	2.7	0.431364
7.9	0.897627	254	0.743301	1.426679	1	0	4.5	0.653213
3.7	0.568202	101	0.664183	0.801713	0.6	-0.22185	2.9	0.462398
9.3	0.968483	109	0.671298	1.982373	1	0	4.7	0.672098
2	0.30103	49	0.590153	0.513898	0.6	-0.22185	1.5	0.176091
12	1.079181	108	0.670444	2.562935	0.3	-0.52288	1	0
6.1	0.78533	152	0.701039	1.214201				
8.4	0.924279	59	0.610398	2.060067	0.6	-0.22185	7.5	0.875061
5.3	0.724276	70	0.628235	1.247501	0.6	-0.22185	1.4	0.146128
12.6	1.100371	97	0.660364	2.754272	0.8	-0.09691	3.7	0.568202
5.4	0.732394	95	0.658381	1.185803	0.6	-0.22185	3	0.477121
3.4	0.531479	81	0.642904	0.773703	0.5	-0.30103	1.3	0.113943
3.2	0.50515	59	0.610398	0.784787	0.6	-0.22185	2.3	0.361728
1.9	0.278754	33	0.543634	0.543399	0.3	-0.52288	0.3	-0.52288
6.7	0.826075	73	0.632504	1.561604	0.6	-0.22185	1.5	0.176091
3.6	0.556303	31	0.535799	1.048344	0.7	-0.1549	2.3	0.361728
7	0.845098	66	0.622178	1.670782	0.5	-0.30103	0.9	-0.04576
4.3	0.633468	93	0.656347	0.948683	0.6	-0.22185	1.7	0.230449
7.9	0.897627	128	0.685924	1.628184	0.8	-0.09691	2.9	0.462398
5.3	0.724276	143	0.695731	1.067936				
4.9	0.690196	75	0.635231	1.134919	0.8	-0.09691	4.7	0.672098
3.1	0.491362	34	0.547327	0.879093	0.3	-0.52288	0.8	-0.09691
10.5	1.021189	193	0.704373	2.074035	0.7	-0.1549	1.7	0.230449
4.4	0.643453	42	0.632504	1.025531	1	0	5.2	0.716003
8.1	0.908485	81	0.721208	1.539137	0.4	-0.39794	3.1	0.491362
3.3	0.518514	32	0.572601	0.882903	0.4	-0.39794	1.3	0.113943
3.3	0.518514	77	0.642904	0.750947	0.8	-0.09691	2.7	0.431364
2.6	0.414973	64	0.539795	0.750201	0.5	-0.30103	2	0.30103
7.5	0.875061	140	0.693871	1.517714	0.7	-0.1549	1.2	0.079181
4.9	0.690196	133	0.68934	1.001973				
2.5	0.39794	78	0.639159	0.573828	0.6	-0.22185	2.3	0.361728
10.2	1.0086	117	0.677805	2.141879	1	0	13.3	1.123852
10.5	1.021189	156	0.703279	2.079267	0.9	-0.04576	9.3	0.968483
7.8	0.892095	101	0.664183	1.690097	0.6	-0.22185	1.8	0.255273
4.7	0.672098	70	0.63787	1.082001	0.5	-0.30103	2	0.30103
4.2	0.623249	149	0.618977	1.009887	0.5	-0.30103	1	0
2	0.30103	132	0.628235	0.470755	0.4	-0.39794	0.7	-0.1549

8.2	0.913814	133	0.699313	1.638707	1	0	4.3	0.633468
7.8	0.892095	73	0.688669	1.597443	1.2	0.079181	14.8	1.170262
6	0.778151	73	0.68934	1.226906	0.7	-0.1549	2.1	0.322219
7.6	0.880814	97	1.986772	0.078351	0.8	-0.09691	0.6	-0.22185
5.6	0.748188	47	1.672098	0.119149	0.8	-0.09691	1.6	0.20412
2	0.30103	19	1.278754	0.105263	1	0	1.8	0.255273
2.1	0.322219	20	1.30103	0.105	0.4	-0.39794	1.2	0.079181
4.9	0.690196	94	1.973128	0.052128	1.1	0.041393	1.6	0.20412
3.6	0.556303	44	1.643453	0.081818	1.2	0.079181	2.6	0.414973
4.9	0.690196	61	1.78533	0.080328	0.8	-0.09691	1.4	0.146128
8.1	0.908485	91	1.959041	0.089011	1.2	0.079181	1.6	0.20412
5.2	0.716003	88	1.944483	0.059091	0.9	-0.04576	1.1	0.041393
4.5	0.653213	27	1.431364	0.166667	0		2.3	0.361728
4.4	0.643453	45	1.653213	0.097778	1.7	0.230449	4.7	0.672098
2	0.30103	38	1.579784	0.052632	0.7	-0.1549	1	0
3.1	0.491362	47	1.672098	0.065957	0.8	-0.09691	2.1	0.322219
14.3	1.155336	66	1.819544	0.216667	1.1	0.041393	2.4	0.380211
1.7	0.230449	39	1.591065	0.04359	1	0	0.9	-0.04576
2.2	0.342423	30	1.477121	0.073333	0.9	-0.04576	1.5	0.176091
2.5	0.39794	64	1.80618	0.039063	1.1	0.041393	0.5	-0.30103
13.9	1.143015	100.1	2.000434	0.139	1.4	0.146128	2.5	0.39794
3.1	0.491362	29	1.462398	0.106897	0.9	-0.04576	0.4	-0.39794
10.3	1.012837	76	1.880814	0.135526	1.7	0.230449	0.8	-0.09691
18	1.255273	70	1.845098	0.257143	1.4	0.146128	0.3	-0.52288
10.9	1.037426	79	1.897627	0.137975	1.4	0.146128	3.8	0.579784
11.6	1.064458	131	2.117271	0.08855	1.4	0.146128	2.6	0.414973
8.9	0.94939	104	2.017033	0.085577	1	0	3.1	0.491362
12.2	1.08636	69	1.838849	0.176812	2.2	0.342423	4.6	0.662758
8.9	0.94939	55	1.740363	0.161818	0.7	-0.1549	1.2	0.079181
11.3	1.053078	65	1.812913	0.173846	1.6	0.20412	1.7	0.230449
10.5	1.021189	100.1	2.000434	0.105	1.3	0.113943	1.4	0.146128
13.2	1.120574	112	2.049218	0.117857	2.3	0.361728	7.5	0.875061
10.1	1.004321	133	2.123852	0.07594	1.5	0.176091	2.3	0.361728
14	1.146128	134	2.127105	0.104478	1.4	0.146128	5.7	0.755875
6.5	0.812913	63	1.799341	0.103175	1.1	0.041393	1.8	0.255273
5.1	0.70757	75	1.875061	0.068	1.1	0.041393	2.3	0.361728
3.4	0.531479	57	1.755875	0.059649	1.3	0.113943	1.4	0.146128
5.9	0.770852	86	1.934498	0.068605	0.5	-0.30103	0.8	-0.09691
13	1.113943	117	2.068186	0.111111	0.6	-0.22185	3	0.477121
6.7	0.826075	159	2.201397	0.042138	3	0.477121	19.6	1.292256
8.1	0.908485	133	2.123852	0.060902	1.2	0.079181	1	0
20.6	1.313867	115	2.060698	0.17913	0.7	-0.1549	1	0
11.2	1.049218	81	1.908485	0.138272	1.8	0.255273	5.3	0.724276
7.9	0.897627	210	2.322219	0.037619	3	0.477121	6.8	0.832509
11.7	1.068186	78	1.892095	0.15	1.2	0.079181	6	0.778151
6.2	0.792392	101	2.004321	0.061386	1	0	2.4	0.380211

2.4	0.380211	29	1.462398	0.082759	0.7	-0.1549	1.8	0.255273
11.3	1.053078	88	1.944483	0.128409	1.1	0.041393	2.5	0.39794
8.4	0.924279	89	1.94939	0.094382	1	0	2.3	0.361728
24.7	1.392697	190	2.278754	0.13	0.5	-0.30103	1.1	0.041393
7.4	0.869232	111	2.045323	0.066667	3	0.477121	7.1	0.851258
6	0.778151	75	1.875061	0.08	0.9	-0.04576	8.7	0.939519
11.3	1.053078	80	1.90309	0.14125	0.8	-0.09691	1.6	0.20412
7.8	0.892095	113	2.053078	0.069027	1.4	0.146128	4.1	0.612784
3.4	0.531479	51	1.70757	0.066667	0.8	-0.09691	0.7	-0.1549
1.9	0.278754	95	1.977724	0.02	1.3	0.113943	1.8	0.255273
7.1	0.851258	53	1.724276	0.133962	0.3	-0.52288	1.5	0.176091
9.4	0.973128	47	1.672098	0.2	0.8	-0.09691	2.2	0.342423
4.3	0.633468	51	1.70757	0.084314	0.8	-0.09691	1.7	0.230449
1.7	0.230449	37	1.568202	0.045946	0.6	-0.22185	0.5	-0.30103
2.5	0.39794	36	1.556303	0.069444	0.5	-0.30103	1	0
7	0.845098	97	1.986772	0.072165	1.6	0.20412	4.4	0.643453
14.7	1.167317	133	2.123852	0.110526	3.4	0.531479	7.9	0.897627
5.9	0.770852	85	1.929419	0.069412	1.3	0.113943	1.9	0.278754
1.8	0.255273	17	1.230449	0.105882	0.8	-0.09691	2	0.30103

R_RPKID	HG	CORTA	LOGCORT	B_A	LOGB_A	MEANA	MEANB_A	SEX
0.25	0.132					19.3	28.46	m
0.156627		4.13	0.61595	21.57	1.33385			m
0.147059		3.68	0.565848	2.32	0.365488			m
		6.95	0.841985	3.11	0.49276			m
0.407407		5.26	0.720986	1.08	0.033424			f
0.222222		7.8	0.892095	62.47	1.795672			f
0.206897		45.1	1.654177	22.3	1.348305			f
0.212766								m
0.4	0.048	4.6	0.662758	17.4	1.240549	6.9	22.7	m
0.3		12.5	1.09691	19.6	1.292256			m
								f
0.08		13	1.113943					m
0.428571		6.01	0.778874	18	1.255273			f
0.216216		53	1.724276					m
0.2		12.2	1.08636	21.8	1.338456			f
0.384615								f
0.26087		8	0.90309					f
1		2	0.30103	3.65	0.562293			f
0.4	0.089	13	1.113943	53.8	1.730782	10	20.93	f
0.304348		7.3	0.863323	-2.95				f
0.555556				12.8	1.10721			m
0.352941	0.129	23.2	1.365488	8.7	0.939519	37.3	15.7	m
0.275862		75	1.875061	23.4	1.369216			m
		14	1.146128					f
0.170213	0.093	4.39	0.642465	2.4	0.380211	8.01	32.27	f
0.375		9.39	0.972666	78.18	1.893096			f
0.411765		5.24	0.719331	3.26	0.513218			f
0.192308								m
0.129032								f
0.307692		5.78	0.761928	17.8	1.25042			m
0.296296		6.97	0.843233	14.86	1.172019			m
0.25		7.75	0.889302	0.95	-0.02228			f
0.583333	0.065	23.65	1.373831	23.7	1.374748	4.49	4	f
		12.68	1.103119	43.2	1.635484			m
0.26087								m
0.075188		7.64	0.883093	0.5	-0.30103			f
0.096774		3.93	0.594393	0.5	-0.30103			f
0.333333		1.9	0.278754	11.1	1.045323			f
0.25	0.156	23.6	1.372912	32.4	1.510545	20.5	47	f
0.5		15.7	1.1959	60.53	1.781971			m
0.571429								m

0.232558		18.3	1.262451	29.1	1.463893			m
0.081081		21.8	1.338456	65.6	1.816904			f
0.333333		19.5	1.290035	47.5	1.676694			f
1.333333	0.0657	9.47	0.97635	55.96	1.747878	32.3	54.3	m
0.5		4.18	0.621176	60.54	1.782042	17.66	42.96	m
0.555556		0.196	0.707744	62.894	1.798609			m
0.333333	0.0592	1.224	0.087781	50.996	1.707536			f
0.6875		12.16	1.084934	30.61	1.485863			m
0.461538		8.05	0.905796	81.22	1.909663			m
0.571429		2.82	0.450249	133.37	2.125058			m
0.75		9.3	0.968483	31.45	1.497621			m
0.818182		32.73	1.514946	43.2	1.635484			m
0		7.65	0.883661	60.19	1.779524	17.56	46.33	m
0.361702	0.0422	1.77	0.247973	97.59	1.989405	3.7	65	m
0.7		1.811	0.257918	38.689	1.587588			m
0.380952	0.0588	0.974	0.011441	61.586	1.789482			m
0.458333		51.37	1.71071	16.44	1.215902			f
1.111111		0.87	0.060481	62.87	1.798443			m
0.6		8.9	0.94939	63.7	1.804139			m
2.2		4.77	0.678518	105.87	2.024773	5.53	68.6	f
0.56		8.59	0.933993	54.93	1.73981			m
2.25		6.21	0.793092	65.78	1.818094			f
2.125	0.0763	3.4	0.531479	46.94	1.671543			f
4.666667		6.79	0.83187	56.45	1.751664	3.33	56.53	m
0.368421	0.0842	1.64	0.214844	84.99	1.929368			f
0.538462		1.79	0.252853	31.3	1.495544			m
0.322581		15.56	1.19201	86.33	1.936162	13.083	44.7	f
0.478261		23.26	1.36661	113.32	2.054307	26.08		f
0.583333		2.19	0.340444	158.85	2.200987			m
0.941176		33.51	1.525174	22.01	1.34262			f
0.928571	0.187	17.38	1.24005	58.08	1.764027			f
0.306667		8.67	0.938019	25.7	1.409933			f
0.652174		13.22	1.121231	50.41	1.702517			f
0.245614	0.216	40.57	1.608205	96.01	1.982316			f
0.611111		22.22	1.346744	180.63	2.25679			m
0.478261		9.27	0.96708	16.55	1.218798			m
0.928571		42.79	1.631342	23.68	1.374382			f
0.625		14.01	1.146438	176.52	2.246794	9.06	109.3	m
0.2	0.0848	4.135	0.616476	90.665	1.95744	43.53	93.4	f
0.153061		7.74	0.888741	63.18	1.80058	5.49	77.26	m
1.2		6.48	0.811575	44.67	1.650016			f
0.7	0.0962	9.11	0.959518	100.6	2.002598			f
0.339623		72.44	1.859978	71.96	1.857091			m
0.441176		49.38	1.693551	10.79	1.033021			f
0.2	0.0835	3.41	0.532754	34.24	1.534534			f
0.416667		5.39	0.731589	78.87	1.896912			m

		4.12	0.614897	52.38	1.719165			f
0.388889		3	0.477121	34.16	1.533518	11.77	84.93	m
0.44		1.27	0.103804	158.243	2.199325	6.11	46.53	m
0.434783	0.0356	0.37	0.431798	55.87	1.747179			f
0.454545		8.99	0.95376	29.19	1.465234			m
0.422535		8.9	0.94939	51.81	1.714414			m
0.103448		1.14	0.056905	70.97	1.851075			m
0.5	0.0392	7.88	0.896526	43.52	1.638689			f
0.341463		1.64	0.214844	67.59	1.829882			f
1.142857		34.86	1.542327	135.47	2.131843			m
0.722222		0.1537	0.813326	64.8963	1.81222			m
0.2		21.09	1.324077	71.63	1.855095	14.1	113.09	m
0.363636		9.26	0.966611	69.786	1.843768	14.27	65.2	f
0.470588		6.2	0.792392	79.75	1.901731			m
1.2		4.1	0.612784	73.26	1.864867			m
0.5		6.39	0.805501	130.81	2.116641			m
0.363636	0.0404	34:94	1.543323	232.86	2.367095			f
0.43038		7.67	0.884795	46.97	1.671821			m
0.684211		1.47	0.167317	59.59	1.775173			f
0.4		1.16	0.064458	168.68	2.227064			m

TIME	BWT	BW2	LIP2	MRK	MRL
am	21.68	19.54		0.457594	0.067114
am	16.94			0.286686	0.056844
am	20.19			0.269173	0.089265
am	18.44				0.093508
am	19.95			0.745709	0.048533
pm	20.44			0.406751	0.056929
am	21.04			0.378699	0.067053
am	17.64			0.389442	0.15617
pm	21.01	20.09		0.732151	0.074709
am	19.51			0.549113	0.203375
am	20.32				0.073456
am	18.05			0.14643	0.260596
pm	18.96			0.784448	0.138586
am	20.92			0.395757	0.23776
am	20.42			0.366076	0.104043
pm	22.02			0.703991	0.076831
pm	19.49			0.47749	0.099275
pm	21			1.830378	0.105385
am	19.65	19.36		0.732151	0.167994
pm	19.05			0.557071	0.21256
am	19.5			1.016877	0.194131
am	20.94	20.25		0.646016	0.08463
am	21.78			0.504932	0.112969
am	18.02				0.067839
am	20.82	20.6		0.311554	0.119585
am	21.97			0.686392	0.166887
pm	19.16			0.753685	0.121639
am	24.42			0.351996	0.110324
pm	19.68			0.236178	0.076819
am	22.96			0.563193	0.143815
am	20.57			0.542334	0.074571
am	18.98			0.457594	0.148718
am	15	19.78		1.06772	0.098056
am	21.18				0.067435
pm	20			0.47749	0.058666
am	20			0.137622	0.159571
pm	22			0.177133	0.123198
am	20.5			0.610126	0.141356
am	19.36	19.74		0.457594	0.111724
pm	19.29			0.915189	0.120119
am	21.47			1.04593	0.052297

am	19.05		0.425669	0.100732
am	21.22		0.148409	0.108159
am	19.31		0.610126	0.082573
am	20	22.49	2.440504	0.143411
am	23.73	22.48	0.915189	0.218088
am	21.14		1.016877	0.192671
am	21.85		0.610126	0.19219
am	24.7		1.258385	0.095413
am	22.92		0.84479	0.149758
am	21.34		1.04593	0.14703
am	21.59		1.372783	0.162924
am	22.75		1.497582	0.108159
am	20.76	20.69		0.305063
am	19.72	22.14	0.662052	0.17897
am	21.59		1.281264	0.096336
am	21.03		0.697287	0.120727
am	24.85		0.838923	0.396582
am	20.55		2.033753	0.079786
am	22.46		1.098227	0.134228
am	20.22	20.36	4.026831	0.071499
am	20.95		1.025012	0.254168
am	18.63		4.11835	0.195661
am	19.99		3.889553	0.248064
am	23.64	21.9	8.541763	0.470669
am	20.26		0.67435	0.252546
am	21.82		0.985588	0.162079
am	21.7	20.52	0.590444	0.156638
am	21.22	21.68	0.875398	0.323632
am	23.57		1.06772	0.296188
am	13.27		1.722708	0.318204
am	17.63		1.699636	0.191998
am	20.51		0.561316	0.215723
am	23.43		1.193725	0.138999
am	20.92		0.449566	0.191233
am	22.44		1.118564	0.188848
am	20.21		0.875398	0.124466
am	20.86		1.699636	0.10918
am	20.59	21.71	1.143986	0.125572
am	19.51	18.85	0.366076	0.203375
am	23.78	23.3	0.28016	0.077129
am	15.85		2.196453	0.111474
am	16.18		1.281264	0.327876
am	22.21		0.621638	0.253089
am	21.49		0.80752	0.068857
am	20.13		0.366076	0.274557
am	26.56		0.762657	0.11236

am	19.78			
am	22.06	23.62	0.711814	0.15148
am	21.84	22.75	0.805366	0.235037
am	23.04		0.795816	0.172755
am	21.09		0.83199	0.237949
am	23.76		0.773399	0.122025
am	22.15		0.189349	0.14643
am	21.83		0.915189	0.258541
am	25.34		0.625007	0.126345
am	24.4		2.09186	0.122025
am	23.42		1.321939	0.036608
am	20.57	21.7	0.366076	0.245202
am	20.44	21.34	0.665592	0.366076
am	21.77		0.861354	0.154326
am	23.79		2.196453	0.084098
am	22.1		0.915189	0.12711
am	22.35		0.665592	0.132089
am	20.27		0.787757	0.202305
am	21.4		1.252364	0.12705
am	21.46		0.732151	0.193805