The Accumulation, Distribution, and Adverse Effects

of Dietary Uranium in Lake Whitefish

(*Coregonus clupeaformis*)

By

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A Thesis
Submitted to the Faculty of Graduate Studies
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THE ACCUMULATION, DISTRIBUTION, AND ADVERSE EFFECTS OF DIETARY

URANIUM IN LAKE WHITEFISH (Coregonus clupeaformis)

BY

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Abstract

Canada is the world's leading uranium (U) producer; mining and milling operations in northern Saskatchewan generate about one third of total global production. These activities discharge U directly into aquatic ecosystems, where it accumulates in sediments. In turn, fish accumulate U via ingestion of sediments and contaminated diet items. However, there is a paucity of data regarding accumulation and deleterious effects of U in freshwater fish. To address these issues, a benthic feeding fish, lake whitefish (*Coregonus clupeaformis*), was fed a commercial diet containing U at concentrations of 100 μg/g, 1000 μg/g, and 10 000 μg/g for 10, 30, and 100 days. The exposure concentrations represent the reported range of U concentrations in sediments of impacted systems in Canada. Measurement of U accumulation in nine tissues revealed bone, scales, intestine, kidney, liver, and gonads accumulated the highest concentrations of U, with no significant accumulation in muscle or skin. The highest concentrations of U in gill and gonad occurred on d 30. Sub-lethal U toxicity was assessed via examination of a suite of biomarkers, spanning several levels of biological organization. Whole organism morphometrics were unaffected by U exposure. Liver somatic index (LSI) was affected in the highest treatment only. Haematological variables were transiently disturbed. U induced metallothionein (MT) in liver of fish fed 10 000 μg U/g, but this response did not persist. Lesions were present in the livers and posterior kidneys of fish from all treatment groups. The frequency and severity of histopathologies were dose- and duration-dependent. Lipid peroxides (LPO) in serum were increased in all treatments on days 30

and 100. The results indicate that diets containing concentrations of U at least as low as 100 μg/g exert sub-lethal toxicity in lake whitefish. The most sensitive and reliable indicators of U exposure and toxicity are: U residues in intestines, liver, kidney, bone, and scales, LPO in serum, and histopathologies in liver and posterior kidney.

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Abbreviations

ATPase	adenosine triphosphatase
BUN	blood urea nitrogen
Bq	Becquerel
cc	cubic centimetre
CF	condition factor
Ci	Curie
cm	centimetre
cpm	counts per minute
CYP	cytochrome P450
d	day
DL	detection limit
DNA	deoxyribonucleic acid
d	day
DO	dissolved oxygen
dw	dry weight
F	female
g	gram
GBM	glomerular basement membrane
GFR	glomerular filtration rate
GSI	gonadosomatic index
Gy	Gray
h	hour
H&E	haematoxylin and eosin stain
HSP	heat shock protein
ICRP	International Commission on Radiological Protection
kBq	kiloBecquerel
kDa	kilodalton
kg	kilogram
L	litre
LC50	concentration lethal to half the subjects in the specified time
LD	lumen diameter
LD50	dose lethal to half the subjects in the specified time
LET	low energy transfer
LOEC	lowest observed effect concentration
LPO	lipid peroxides
LSI	liver somatic index
M	male
mBq	milliBecquerel
MCHC	mean corpuscular haemoglobin concentration
MDA	malondialdehyde
meq	milliequivalent
mg	milligram
mGy	milliGray

min	minute
mL	millilitre
mm^3	cubic millimetre
mM	millimole
mOsm	milliosmole
mRNA	messenger ribonucleic acid
mSv	millisievert
MS-222	tricaine methane sulfonate
MT	metallothionein
N : C	hepatocyte nuclear area to cytoplasm area ratio
nmol	nanomole
NOEC	no observed effect concentration
P1	first segment of the proximal tubule
pCi	picoCurie
P2	second segment of the proximal tubule
PCV	packed cell volume
PM	pigmented macrophage
PMA	pigmented macrophage aggregate
ppm	parts per million
PUFA	polyunsaturated fatty acids
r	radius
RER	rough endoplasmic reticulum
RNA	ribonucleic acid
rpm	revolutions per minute
rRNA	ribosomal ribonucleic acid
SEM	Saskatchewan Energy and Mines
SE	standard error of the mean
SOD	superoxide dismutase
Sv	sieverts
TCA	trichloroacetic acid
TD	tubule diameter
TDN	total dissolved nitrogen
TEH	tubule epithelium cell height
μCi	microCurie
μeq	microequivalent
μg	microgram
μL	microlitre
μm	micron
μm^2	square micron
WBW	wet body weight
ww	wet weight
yr	year

Chapter One

Introduction: U in the aquatic environment and mammalian

toxicology.

I. U Mining and Milling

Due to substantial uranium reserves in the province of Saskatchewan, Canada is the world's leading uranium producer. Northern Saskatchewan contains among the world's richest and largest U ore deposits discovered thus far. Activities in this province generated 11 405 t of U in 1996 and an estimated 12 021 t in 1997; annual productions that equal approximately one third of total world U production (SEM 1998). Existing and new mining operations in this area include: the McClean Lake Project, the Cluff Lake Mine, the Key Lake Mine, the Rabbit Lake Mine, and the McCarthur River and Cigar Lake operations. These deposits are estimated to contain sufficient quantities of ore to maintain operations for at least 25 years. Furthermore, the McCarthur River deposit, one of the richest U sources in the world, is believed to contain 72 700 t of uranium deposits at an average grade of 15.9% U_3O_8 (SEM 1998). In the past, most mined ore deposits typically contained 1% U or less, and more frequently between 0.025 and 0.25% (Chambers et al. 1989).

Saskatchewan also contains several of Canada's abandoned (not monitored) and inactive (still subject to monitoring) waste sites resulting from decommissioned mining and milling operations (Kalin 1988). These operations include: the Beaverlodge Lake facility that ceased operation in the early 1980's, the Lorado operation that discharged 0.5 million t of tailings into Nero Lake, and Gunnar Mines which deposited 5 million t of tailings over a 65-hectare area forming a small beach on Lake Athabasca. The latter two operations ceased production in the 1960's (Kalin 1988).

U contamination is of concern in aquatic environments nearby U mining and milling operations, because these activities release U directly into freshwater ecosystems. U mining and milling activities release more U to the environment than any other point in the nuclear fuel cycle, despite extremely efficient extraction processes (Poston 1982). Although great attention is paid to the highly radioactive daughter progeny of U, as well as many of the metals and metalloids associated with the mining and milling of U, there remains a paucity of data on U toxicology in aquatic biota.

Hynes (1990) reported that U and Mo were the most important contaminants of concern in systems receiving effluents from the Cluff Lake mine and mill. In only 6 years of effluent discharges, 12 816 kg of U were released into a nearby creek, by far the greatest amount of any of the 19 metals and metalloids or 5 radionuclides measured. The greatest quantity of U was released during the years of high grade ore processing. A nearby lake, Island Lake, receiving these discharges contained an estimated 4048 ± 1810 kg (mean ± SD) U following 6 years of effluent discharges. The large quantity of U released into this system occurred despite consistent compliance with both federal and provincial effluent discharge limits (Hynes 1990). Clearly, as proved by the Cluff Lake example, U mining and milling activities cause U enrichment of nearby aquatic systems.

U mining and milling activities release U into the environment at several points and in several forms throughout their operation. Aquatic ecosystems are particularly at risk, as U enters these systems directly via effluents, mine dewatering discharges, aerial particulate deposition from tailings erosion (Breslin and Glauberman 1970) and mine exhaust air (Bunzl et al. 1994), ground water contamination (Moffett and Tellier 1978,

Veska and Eaton 1991), and dissolution of tailings following precipitation and surface water runoff (Kalin 1988, Moffett and Tellier 1978, Waite et al. 1989).

Typically, the abiotic environments nearby U mining and milling operations are enriched in U. Researchers have reported elevated concentrations of uranium in surface waters, ground water, soil, and sediments of aquatic and terrestrial ecosystems near these sites. In the aquatic environment, U accumulates primarily in the sediment compartment, which effectively acts as a U sink (Hynes 1990). Concentrations of U in the sediments of Canadian aquatic systems are summarized in Appendix two. Concentrations reported in enriched global systems are provided in Appendix three.

Although surface waters and groundwaters are not the main sites of U accumulation in aquatic ecosystems, they may contain elevated concentrations as a result of mining and milling. For instance, a stream near Gunnison, Colorado, receiving U mining discharges and natural U inputs, contained concentrations ranging up to 4 mg/L (Parkhurst et al. 1984). High concentrations of U may occur because of its high solubility in oxic waters. In its stable hexavalent state (i.e. U^{6+} or $(UO_2)^{2+}$), U readily complexes with carbonates, phosphates, and sulfates (Gascoyne 1992). This complexation results in high geochemical mobility and U may be transported over large distances (Cowart and Burnett 1994).

Despite the high solubility of U in surface waters (Cowart and Burnett 1994), the sediments accumulate the highest concentrations and the greatest burden of U in freshwater ecosystems. For instance, Hynes (1990) estimated that the sediments of Island

Lake contained 36 ± 16% of the total discharged U. For comparison, surface waters contained only 14%. The remaining U was transported downstream (Hynes 1990).

The surficial sediments of systems impacted by U mining and milling contain concentrations of U ranging from < 100 µg/g to > 1000 µg/g (dw) (Hynes 1990, Hynes et al. 1987, Joshi et al. 1989, McKee et al. 1987, Swanson 1985). Concentrations as high as 5650 µg/g (dw) were reached in the upper two centimetres of sediments of the Wollaston Lake watershed, contaminated by U mining and milling activities (Neame et al. 1982). The highest concentration reported for Canadian sediments, 18 000 µg/g (dw), occurred in Port Hope Harbour, Lake Ontario, near the site of a U refinery (Hart et al. 1986).

Elevated concentrations of U in sediments persist in impacted systems on the order of decades, or longer. For example, the upper strata of sediment cores taken from Langley Bay, Lake Athabasca in 1983, 25+ years following the decommissioning of the Gunnar Uranium mine and mill complex, contained a concentration of 300 µg U/g (dw) (Joshi et al. 1989).

II. Other Sources of U Enrichment

Although the impetus behind this study is largely rooted in the activities of U mining and milling, U is also released to the environment from several other anthropogenic sources. These activities include discharges from other points in the nuclear fuel cycle (Rao et al. 1996), phosphate mining and large scale application of phosphate fertilizers (Bradford et al. 1990, Guimond and Hardin 1989), and the combustion of fossil fuels (Beck 1989, Burkhart 1991, Cowart and Burnett 1994).

Additionally, isolated contamination of aquatic systems may occur nearby U research or manufacturing facilities (Emery et al. 1981, Keklak et al. 1994). Finally, although rare, accidents at nuclear facilities, such as Chernobyl (Jeschki 1989) and Tomsk-7 (Porfiriev 1996), may contribute substantially to releases of U to the environment.

Although less significant than the latter sources, depleted U is also released to the environment with its use as military ammunition. This is a very recent source of U in the global environment but may be of concern in localized areas (e.g. Iraq) where depleted U bullets are used extensively. Some suspect this U to be the cause of widely reported health problems, the so called 'Gulf War Syndrome', experienced by American soldiers and the Iraqi public (Doucet 1994). However, impacts of U ammunition to the aquatic environments are unknown. With the exception of the latter, all of the activities listed above are known sources of surface water contamination.

Phosphate rocks contain between 3 and 400 μg U/g (Mortvedt 1994), generally higher than the estimated mean concentration, 3 $\mu g/g$, in the earth's crust (Bosshard et al. 1992). Consequently, phosphate fertilizers are relatively U-rich (Hussein 1994, Mortvedt 1994). Phosphate fertilizers produced in Finland contained concentrations of ^{238}U ranging from 980 to 7400 Bq/kg, equivalent to approximately 78.7 and 594.4 mg/kg, respectively. There is sufficient U in phosphate fertilizers for some manufacturers to remove U and sell it as yellowcake (Mortvedt 1994).

The production of phosphate fertilizers releases U into the aquatic environment. Contributions of U from these practices are substantial enough to have caused increased residues in aquatic biota. For example, concentrations of U in the digestive gland of

American lobster (*Homarus americanus*) inhabiting an industrialized harbour in New Brunswick, Canada, were greatest, approximately nine times the mean of reference lobsters, in lobsters sampled near the outfall of a phosphate fertilizer plant (Chou and Uthe 1995).

Following large-scale and long-term application of fertilizers to agricultural land, U accumulates in the soil and crops (Mortvedt 1994). Evaluations of heavily fertilized regions in the U.S. illustrate the magnitude of this source of pollution. It was estimated that in some potato fields in Maine, 3.7×10^7 Bq of U, equivalent to approximately 1.480 kg U, were applied to each hectare of land over a 45 year period (Mortvedt 1994).

U originating from agricultural practices enters aquatic ecosystems by soil erosion, irrigation, and surface water runoff following precipitation (Bradford et al. 1990, Buhl and Hamilton 1996, Guimond and Hardin 1989). The San Joaquin Valley, California, an area under intensive agricultural development, serves as an example of the degree of potential contamination from this source. Mean concentrations of U in saline evaporation ponds of this region were 896 µg/L, although concentrations reached as high as 9900 µg/L (Bradford et al. 1990). Contamination from agricultural irrigation return waters in the western U.S. is serious enough to have prompted recent evaluations of U toxicity to a native fish species (Hamilton and Buhl 1997). Because the use of phosphate fertilizers will likely persist, this source of U may become an increasing environmental concern in the future.

Fossil fuel combustion is likely to be primarily a global concern as it may result in chronic, elevated inputs of U to 'pristine' freshwater ecosystems following long-range

atmospheric transport. Concentrations of U in coal vary by four orders of magnitude worldwide; ranging from < 1 μg/g to 1800 μg/g (North Dakota lignite) (Tadmor 1986). Coal fly-ash from power plants in Hungary contained U concentrations as high as 162 μg/g (Rausch et al. 1993). Furthermore, because large volumes of fossil fuels are burnt, appreciable quantities of U, and other radionuclides, are released into the global environment even where the fuel contains little U (i.e. 1 μg/g) (Okamoto 1980). In addition, localized impacts can occur, for instance, in aquatic systems adjacent to coal-fuelled power stations. Elevations in the concentrations of U in water and resident fish have been observed in systems neighbouring these operations (Sarosiek et al. 1991).

III. Uranium Toxicology in Mammals

Although an exhaustive review of the toxicology of U in mammals is beyond the scope of this discussion, a brief overview of the accumulation and adverse effects of U is warranted in light of the absence of similar data in fish. The following discussion is a synopsis of the literature with a particular emphasis placed upon dietary uptake studies.

A. U Accumulation in Mammals

The mammalian biokinetic model for uranium was recently revised, largely motivated by the Chernobyl nuclear accident. The current model is based upon the framework applied to the alkaline earth elements, due to similarities in the behaviours of these elements in skeletal tissues (Leggett 1994). U and the alkaline earths function, in some capacity, as calcium antagonists in mammals.

The main sites of U accumulation in mammals, including humans, are the kidney and bone, and to a lesser degree, liver (Leggett 1994). The greatest burden of U is retained in skeletal tissues. The most significant route by which humans and non-human mammals are exposed to U is ingestion of contaminated food and drinking water.

The International Commission on Radiological Protection (ICRP) accepts that 2% of ingested U is absorbed across the gut and into the circulation (i.e. gastrointestinal transfer coefficient) (Leggett 1994). The majority of U in the circulation is rapidly filtered in the kidney and excreted with the urine. Of the fraction retained in the body, most is deposited in bone and kidney.

i. Bone

U is a well established bone-seeker in mammals (Harrison and Stather 1981, La Touche et al. 1987, Sullivan and Ruemmler 1988, Sullivan et al. 1986, Tracy et al. 1992), including humans (Fisenne and Welford 1986, Kathren et al. 1989, Singh et al. 1987a, b, Welford and Baird 1967). In mammals, 15% of U leaving the blood is deposited on the surfaces of bones (Leggett 1994). In reference man, the human model for non-occupationally exposed individuals, 66% of the U body burden is contained in the skeleton (Wrenn et al. 1985). In agreement, using their model for chronic exposures to U, the ICRP predicted a value of 75% (Leggett 1994). For comparison, the latter value is equivalent to approximately 30 times the U content of liver (Leggett 1994).

Although U deposits primarily on bone surfaces by substituting for Ca within the hydroxyapatite crystal lattice, it has been recently treated as a bone volume seeker by the

ICRP (Leggett 1994), because it slowly penetrates bone volume (Rowland and Farnham 1969, Stevens et al. 1980). Furthermore, U does not accumulate homogeneously in all bones (Hamilton 1972, Singh et al. 1987b, Welford and Baird 1967). Rather, it is preferentially deposited in bones with a high surface area, high vascularity, and those with high metabolic activity (Stevens et al. 1980). This latter tendency may explain observations of higher U concentrations in the bones of children than adults (Lianqing and Guiyun 1990).

U deposited on bone surfaces is rapidly exchangeable (5 d), whereas U which has diffused into bone volume, or U residing on bone surfaces accreted by deposition of overlying layers of bone, is not (days to years). Long-term mobilization of U from skeletal tissues occurs by bone resorption (Leggett 1994).

ii. Kidney

A substantial database on mammals both confirms the kidney as a principle site of U deposition and helps to delineate the mechanisms of this accumulation. Once taken up into the blood of beagles, U associates with, and equilibrates between, two primary fractions, transferrin and other proteins, and low-molecular-weight anions, primarily carbonates (Stevens et al. 1980). Only the uranyl-carbonate pool is filterable across the glomerulus, but because approximately 60% of U in the circulation exists as this complex, U is rapidly filtered into the urine (Stevens et al. 1980). In mammals, approximately 75% of U in the circulation is filtered and excreted by the kidney within 24 hours (Leggett 1994).

However, a much greater fraction of U than initially thought may associate directly with red blood cells. For example, between 10 and 80% of U in the blood of baboons was bound to red blood cell membranes (Leggett 1994). Using the recently revised ICRP biokinetic model for U, under chronic exposures, an estimated 45% of U in the human circulation would exist in the red blood cell fraction (Leggett 1994).

Not all U that enters the lumens of nephrons is discharged in the urine. Because of the increasingly acidic environment encountered along the length of the proximal tubules, the uranyl-carbonate complex becomes increasingly unstable and dissociates (Leggett 1989). Once released from this ligand, the uranyl ion may interact with the brush border membrane of proximal tubules (Dounce 1949). Because U exists in biological tissues as the positively charged uranyl ion, UO_2^{2+}, it has a strong attraction for anions. This characteristic is manifested as a tendency to complex with the anionic components of the proximal tubule brush border membranes, particularly the phosphate groups of phospholipids (Pasquale et al. 1986).

This affinity for phosphate groups is the basis for some histochemical procedures. Uranyl acetate is used extensively to stain the cellular and subcellular membranes of biological tissues for electron microscopy. It is because U has a specificity and affinity for phosphates and membranes, particularly collagen, nucleic acids, and cell organelles with a high nucleotide content (Payne et al. 1983), and is impermeable to the intense electron beam (Gray 1973), that it is so widely used. Furthermore, uranyl ions have been applied as biological fixatives (Terzakis 1968). Although the precise mechanism of this

fixation is unknown, it may be due to the high affinity of U for DNA and its general ability to stabilize these substances (Stoeckenius 1961).

Virtually all U which accumulates in mammalian kidneys is present in the proximal tubules. Accumulation in these structures has been illustrated by autoradiographical demonstration of U isotopes with a high specific activity in mammals, including humans (Neuman 1949, Stevens et al. 1980). The mode of entry into tubular epithelium has not yet been clearly established, however, two mechanisms of uptake that have been suggested are endocytosis (Leggett 1989) and resorption with water and electrolytes (Galle 1974).

Within the tubular epithelium and in macrophages, U accumulates primarily in lysosomes in the form of an insoluble phosphate crystal (Galle 1974, Galle et al. 1992). The mechanism of this accumulation in both cells appears to be the induction of lysosomal acid phosphatase, an enzyme which liberates phosphates from a variety of substrates (Galle et al. 1992). When accumulation in this organelle is high, the microneedle precipitate pierces the lysosomal membrane and the contents, including the U, are spilled into the cytosol (Galle 1974).

Refining estimates of the fractional uptake, retention, and biological half-time of U in mammalian kidneys has been the focus of U researchers for many years. Reference man is estimated to contain only 0.3% of the total U body burden in the kidneys (Wrenn et al. 1985). Until recently, it was estimated that 11% of·U entering the blood is accumulated in the kidneys, with a residence half-time of 15 days (Wrenn et al. 1985). More currently, these estimates have been revised considerably and a number of

additional components have been added to the model. U accumulation in kidney is now viewed to occur in three tissue compartments, with removal half-times ranging from 4 hours to 5 years (Leggett 1994). What these revisions indicate, and the reason for their modification, is that under chronic exposures to U, there is prolonged retention in this tissue.

iii. Liver

The ICRP accepts that 1.5% of U in mammalian blood is deposited in liver (Leggett 1994). However, this short-term pool (removal half-time = 7 d) is rapidly lost, primarily to plasma. A small fraction is deposited in a long-term component of liver (removal half-time = 10 yr). Thus, the mammalian model indicates that only a small fraction of ingested U accumulates in liver, however, some long-term retention is expected. Experimental research in mammals supports these assumptions. Beagles injected with U contained 1.1% of the injected dose in the liver one day post-exposure. One year later, this value decreased to 0.11% (Stevens et al. 1980). With increasing post-injection time, an increasing percentage of U in the liver was associated with the mitochondrial and lysosomal fractions. On day 726 post-injection, 88.4% of hepatic U was located in these pools (Stevens et al. 1980).

iv. Gonads

Less attention has been placed upon U accumulation in gonads than in other tissues of human and non-human mammals. In the current ICRP biokinetic model for U,

gonads are included in the compartment termed 'massive soft tissues' which incorporates all tissues except blood, bone, kidney, and liver (Leggett 1994). Retention of U in the massive soft tissues may be considerable under chronic exposure; an estimated 20% of total body burden may be located in the massive soft tissue compartment of an adult human (Leggett 1994). For example, beagles injected with ^{233}U accumulated little U in gonads, however, U deposited in this tissue was subject to high retention (Stevens et al. 1980). The estimated residence half-time for U in gonads was 173 days, twice that estimated for kidney. The presence of pools of U subject to long-term retention has been incorporated into the current mammalian biokinetic model for U. The ICRP model accepts that accumulation in massive soft tissues occurs in three compartments, where U located in the longest component has a removal half-time of 100 years (Leggett 1994). Thus, under chronic exposure, a substantive quantity of U is expected to reside in this pool.

B. U Toxicology in Mammals

i. Guidelines for U

As the kidneys are the critical target tissue for the chemotoxicity of U, the principle mechanism of U toxicity, intake limits and drinking water quality guidelines are based upon thresholds of adverse effects in this tissue. Currently, the ICRP accepts that the critical concentration of U in mammalian kidney is 3 μg U/g (Spoor and Hursh 1973). However, contemporary researchers generally concur that this value should be decreased

to 1 μg U/g (Diamond 1989, Leggett 1989, Morris and Meinhold 1995, Wrenn et al. 1985).

Concentrations even lower than the recently advocated safe limit of 1 μg U/g kidney, have been associated with significant renal pathologies in experimental animals as early as 50 years ago (e.g. Hodge 1953). Initially, the threshold concentration for U in mammalian kidney was arbitrarily assigned on the basis of the occurrence of severe histopathologies. Hodge (1953), a researcher for the Manhattan Project, in fact states: "as a guess, 2-3 μg/g might be deposited without serious tissue damage."

As contemporary researchers have pointed out, "serious tissue damage" is not an acceptable endpoint for establishing limits to prevent U toxicity (Morris and Meinhold 1995, Stopps and Todd 1982). Furthermore, as Morris and Meinhold (1995) asked: "At what point should an "effect" be considered "significant?". As they continue to suggest, reducing the "reserve capacity" of the kidney constitutes an adverse effect, as it would effectively impair kidney functioning in the event of an independent challenge. Clearly, the best guess of 2 to 3 μg U/g kidney is not representative of data which has been acquired since 1953, nor is this concentration appropriate considering advances in the sensitivities of technologies and approaches currently applied in evaluating U toxicity.

The Canadian drinking water quality guideline for U is 100 μg/L, increased from an earlier limit of 20 μg/L (Health and Welfare Canada 1993), in accordance with advocations by researchers (Wrenn et al. 1985). Suggested daily intake limits, or the no observed effect limit, to prevent chemical toxicity of U in the kidney of mammals are

approximately 1 mg/kg bw/d (Bosshard et al. 1992), assuming the critical concentration of U in kidney is 1 µg/g.

The relatively recent increase in the acceptable concentration of U in Canadian drinking water has prompted research into the hazards associated with chronic ingestion of U in water, an area which has received relatively little attention by U researchers in the past. A recent epidemiological study of residents of Saskatchewan, a province where drinking water supplies in many regions contain higher than national average concentrations of U, indicated that chronic ingestion of concentrations of U lower than 100 µg/L may produce nephrotoxicities (Mao et al. 1995). A significant positive correlation between the ingestion of U in drinking water (measured as concentration, ingestion volume, and length of residence at the location) and concentrations of urine albumin (i.e. microalbuminuria) was reported. Furthermore, several individuals displayed serum creatinine concentrations consistent with prevalent renal damage.

ii. U Toxicology

A distinction must first be drawn between the chemotoxicity and radiotoxicity of naturally occurring U isotopes in biological systems. Although U is a radionuclide, and in fact heads two of the three naturally occurring decay-chains on earth, its adverse effects on organisms are largely a result of its chemical action and not radiation (Wrenn et al. 1985). Thus, U behaves as a heavy metal and is treated as such in mammalian toxicology and in the present study.

U is cytotoxic (Lin et al. 1993), nephrotoxic (Leggett 1989), genotoxic (Lin et al. 1993, Prabhavathi et al. 1995), embryotoxic (Bosque et al. 1993, Domingo 1994, 1995), teratogenic (Bosque et al. 1993, Domingo 1995), mutagenic (Au et al. 1996, Prabhavathi et al. 1995), and carcinogenic (Terzakis 1995). The specific subcellular sites of chemotoxic action and the mechanisms of toxicity are fairly well documented.

The effects of U on the cell begin at the exteriour where it damages cellular membranes. It is generally agreed that U is a potent membrane toxin (Leggett 1989), however, the precise target sites within the cellular membrane and the mechanisms for membrane toxicity have yet to be fully elucidated. Current information indicates U disrupts proximal tubule resorptive processes and is suspected to alter proteins and inhibit enzymes located in the membranes of proximal tubules (Leggett 1989). In turn, membrane damage is perceived as an important step in the development of tubular cell injury, which may lead to increased Ca uptake, disruptions in electrolyte transport, and possibly alter hormone-cell interactions via damage to membrane phospholipids (Leggett 1989).

Effects on organelles are primarily realized in lysosomes, mitochondria, nuclei, and rough endoplasmic reticulum (RER), with which U has a particular affinity (Galle 1974, Leggett 1989, Stevens et al. 1980, Tasat and de Rey 1987). U accumulates in lysosomes as an insoluble mineral precipitate (Galle 1974), and may cause lysosomal proliferation and lysosomal membrane damage (Galle 1974, Tasat and de Rey 1987). U may cause numerous pathologies in mitochondria, including calcification (Carafoli et al. 1971) and various histological alterations (Carafoli et al. 1971, Leggett 1989). Actions on

the RER include fragmentation of polypeptides, disruption of secondary and tertiary protein configurations (Chassard-Bouchaud 1983), and inhibition of enzymes, including microsomal ATPase (Nechay et al. 1980).

In the nucleus, U alters the structure and function of DNA. Effects include mutagenic alterations of DNA repair responses (Au et al. 1996) and alterations in the number and structure of chromosomes (Au et al. 1995, Lin et al. 1993). There is also some evidence that U binds directly to DNA, where it inhibits mRNA production and protein synthesis (Ghadially et al. 1982). DNA and other proteins may also be damaged through the actions of U on lysosomal membranes. With continued accumulation in these organelles in the form of a microneedle phosphate crystal, the crystal may pierce the lysosomal membrane releasing the contents into the cytosol (Galle 1974). Because the lysosomes house proteolytic enzymes, their release into the cytosol may compromise the integrity of cellular proteins.

The primary target tissue for the chemotoxic action of U is the kidney, where it causes numerous structural and functional alterations (Leggett 1989). Structural damage is most pronounced and consistent in the proximal tubules, the main site of U toxicity, however other segments of the nephron may also be affected, including the glomeruli (Bentley et al. 1985, Domingo et al. 1987, Griswold and McIntosh 1973, Kato et al. 1993, McDonald-Taylor et al. 1992).

Functional damage is manifested as proteinuria (Bentley et al. 1985, Hursh and Spoor 1973, Mao et al. 1995), catalasuria (Dounce et al. 1949), glucosuria (Hodge et al. 1973, Voegtlin and Hodge 1949, 1953), and aminoaciduria (Bentley et al. 1985, Voegtlin

and Hodge 1949, 1953). Excessive urinary output of these substances arises by two routes common to all nephrotoxins. Structural damage to the glomerulus allows the passage of macromolecules, that would otherwise be prevented from crossing the membrane owing to their large size, into the ultrafiltrate. Damage to proximal tubules impairs the resorptive capacities of these segments, thus augmenting excretion of macromolecules (Bernard and Lauwerys 1991). Lysosomal enzymes, such as acid phosphatase and ß-galactosidase, may be excessively excreted in the urine as a result of structural damage to the nephron (Stroo and Hook 1977). Furthermore, there is some evidence that damage to the nephron also occurs in segments beyond the proximal tubules, resulting in decreased sodium resorption in distal segments (Bowman and Foulkes 1970).

The main endpoint of renal damage is a decreased nitrogenous waste output arising from a reduction in glomerular filtration rate (GFR). Clinical signs of reduced GFR in mammals exposed to U are increases in blood urea nitrogen (BUN) and/or serum creatinine (i.e. azotemia) (Bentley et al. 1985, Domingo et al. 1987, Stevens et al. 1980, Stroo and Hook 1977). Decreased GFR is correlated to glomerular morphological alterations and may be severe enough to induce acute renal failure (Kato et al. 1993, Pavlakis et al. 1996). Renal impairment induced by acute doses of U persists for at least 6 months in humans (Pavlakis et al. 1996). The main effect of U on interstitial tissue is nephritis, a characteristic of U toxicity exploited as a means of experimentally inducing nephritis for medical research (Barnett and Metcalf 1949, Hursh and Spoor 1973).

Haematological alterations may arise as a result of U intoxication, however, reports on the effects of U on red cells and blood oxygen carrying capacity are

inconsistent. U hemolyses erythrocytes *in vitro* by interacting with the cellular membrane (Stuart et al. 1979). However, U has a biphasic effect on phosphate metabolism in erythrocytes, being stimulated at low doses and inhibited at higher ones (Prokes et al. 1991). Some researchers observed decreased haematocrits (Domingo et al. 1987) or anaemia (Pavlakis et al. 1996), some found no change (e.g. Maynard and Hodge 1949), and still others reported increased haematocrits, haemoglobin, and mean corpuscular haemoglobin concentration (Ortega et al. 1989) in U-intoxicated mammals. Other haematological alterations include increased glucose and protein, and either increased or decreased GOT and GPT in plasma (Bentley et al. 1985, Ortega et al. 1989).

A number of other pathologies have been documented in mammals exposed to U, though subject to less intensive study. These effects include disruptions of the nervous system and reproduction, although these aspects of toxicity have received little attention and additional research has been advocated (Stopps and Todd 1982). It has been reported that U produces a biphasic stimulatory/inhibitory effect on nerve transmission, mediated by disturbances of Na permeability (Lin-Shiau et al. 1979). Of the little research conducted on the effects of U on reproduction, evaluations conducted as part of the Manhattan Project (1942-1945) indicate that adverse effects do occur (Maynard and Hodge 1949). A reduced reproductive output was observed in rats one year following a single, 24-hour exposure to U in food (Maynard et al. 1953). There was also some indication in a second study, that U may disrupt the endocrine system. Female rats fed U contaminated diets displayed irregular oestrus cycles, however, these observations were inconclusive due to concomitant weight loss (Maynard and Hodge 1949).

Other effects of U on the endocrine system have begun to surface in the literature. An epidemiological study of Namibian U miners, which had been found to suffer from a high prevalence of cancer, indicated a highly significant reduction in serum testosterone associated with reduced neutrophil counts, increased chromosome aberrations, and high concentrations of U in urine (Zaire et al. 1997). A second study of laboratory rats indicated that both hot (i.e. neutron-activated) and cold (i.e. nonactivated) nuclear fuel particles (UO_2) markedly reduced rat liver microsomal testosterone hydroxylase activities (Pasanen et al. 1995). This effect persisted one and a half years following a single intratracheal instillation of U.

Actions of U on enzymes and proteins *in vitro* were also studied as part of the Manhattan Project (Dounce and Lan 1949). Those that were precipitated by U without the presence of salts were: egg albumin, serum globulin, human erythrocyte catalase, and urease. Serum albumin, hemoglobin, cytochrome C, and histone were precipitated in the presence of salts. Precipitation appears to occur via the formation of a uranyl ion bridge between the carboxyl groups of adjacent proteins.

Furthermore, any enzyme which contains a phosphate group could complex with U, and possibly become inhibited (Dounce and Lan 1949). For instance, highly U-sensitive enzymes include thrombin, which participates in blood clotting, and phosphorylase, the catalyst for glycogenolysis, both of which contain phosphate groups (Dounce and Lan 1949). Of the two, the inhibitory action of U could only be reproduced *in vivo* for the latter. U also inhibits mammalian ATPases, likely acting directly upon the Na^+ site on the enzyme (Nechay et al. 1980). Furthermore, citric acid augmented this

inhibitory response possibly via reduction of U^{6+} to U^{4+}. Conversely, other enzymes not affected by U *in vitro* are markedly depressed *in vivo*. These include, alkaline phosphatase, δ-amino acid oxidase, and catalase in mammalian kidney (Dounce and Lan 1949).

An observation which has been extensively documented in mammals exposed to U is the development of resistance to this contaminant (Haven 1949, Hodge 1973, Yuile 1973). Two general methods have been used to demonstrate that experimental mammals acclimate to U. In the first, a series of sub-lethal doses of U are given to the animal by any route and tolerance is tested by administering a calculated lethal dose. In the second, a series of increasing doses of U are given to the subject until the calculated lethal dose is reached or exceeded. Both methods have shown that rats, rabbits and dogs demonstrate this response.

It should also be cautioned that although the main toxicological hazards associated with U arise via chemical mechanisms, long-term exposure to and accumulation of U in skeletal tissues may pose a significant radiotoxic risk; primarily an increased risk of developing osteosarcoma (Mays et al. 1985, Stevens et al. 1980, Wrenn et al. 1985). As the issue of radiation is treated in detail in Appendix five, no further discussion is warranted here.

IV. U in Aquatic Biota

The contamination of the abiotic components of aquatic ecosystems is also manifested in the resident biota. Generally, concentrations of U in aquatic biota descend

with each successive trophic level, typically being greatest in algae and plankton and lowest in fish (Mahon 1982, Stegnar and Kobal 1982, Swanson 1982). Although accumulation of U in biota residing in impacted systems has been established for some time, there remains a scarcity of data on concentrations in aquatic organisms. Even less is known regarding seasonal fluctuations of U in the biotic and abiotic components.

Although fish residing in impacted systems are exposed to U via a number of routes, the most significant transfer is achieved via ingestion of sediments and contaminated food (Emery et al. 1981, Kovalsky et al. 1967, Swanson 1982, 1983, 1985). Fish species that occupy a benthic trophic position, which feed upon benthic organisms and have a high degree of association with the sediments, accumulate the highest concentrations of U, relative to pelagic and predatory species (Emery et al. 1981, Kovalsky et al. 1967, Swanson 1982, 1983, 1985). Of the latter two classes, pelagic feeders accumulate more U than top predators (Rao et al. 1996).

The importance of sediment contact and ingestion, and thus fish trophic status, was demonstrated by an artificial caging experiment in a nuclear waste pond on the Hanford Site (Emery et al. 1981). At the time of study, U-Pond contained 5300 kg of ^{238}U at a mean concentration of 650 μg U/g (dw) in the upper 10 cm of sediments. Caged bluegill sunfish (*Lepomis macrochirus*) consistently accumulated higher concentrations of U than caged largemouth bass (*Micropterus salmoides*) throughout a three week exposure and following 60 weeks of exposure. Furthermore, groups of fish from both species that were in direct contact with the sediments and allowed to feed from them, accumulated greater concentrations of U than those isolated from the sediment and fed

uncontaminated artificial food. From these experiments, it was concluded that the transfer of U to fish was via direct contact with the sediments. In addition, the sediments themselves were deemed the most significant source of U, not the fish food. In turn, accumulation of U in fish was directly related to the frequency and duration of sediment contact.

Poston (1982) stated that fish have a low ability to accumulate uranium, a misleading conclusion based upon whole body concentrations achieved in rainbow trout exposed to waterborne U. This conclusion is not unexpected, given the design of this experiment, because analysis of whole fish will greatly underestimate accumulation in the various tissues for which U has an affinity. Although this principle is widely appreciated, Poston's misleading conclusion is still cited in the literature (Ribera et al. 1996). Monitoring of feral fish in freshwater ecosystems is often limited to examinations of U in whole body and/or muscle (Emery et al. 1981, Joshi 1984, Lambrechts and Foulquier 1987, Lucas et al. 1970, Rao et al. 1996, Sarosiek et al. 1991, Stegnar and Kobal 1982, Uthe and Bligh 1971), a practice which fails to appropriately assess the issue of U accumulation. Inevitably, this leads to the potentially erroneous conclusion that fish are not accumulating appreciable quantities of U.

Measurements of contaminant tissue residues are among the most widely applied tools in environmental biomonitoring programs. However, to accurately assess the biological availability of U to fish in contaminated habitats, it is essential to measure U in appropriate tissues. Ideally, analysis of contaminant residues in all freshwater fish tissues would provide sensitive and reliable information regarding exposure and bioavailability

in a temporal and spatial scale and, potentially, information regarding the primary route of uptake. Furthermore, the identification of the main sites of assimilation in fish could indicate potential target organ systems for U toxicity.

Information regarding accumulation and distribution of U in freshwater fish is limited. Available data are derived from a small number of studies of feral fish, which indicate that the primary sites of U accumulation are bone, gastrointestinal tract, kidney, and liver (Swanson 1985, Waite et al. 1988). Although valuable contributions to research in this area, a number of critical tissues were not analyzed in these studies or data were inadequate for revealing trends. No data are available on U accumulation in the tissues of freshwater fish exposed in a controlled laboratory environment, other than rare analyses of whole body concentrations following aqueous exposures (Parkhurst et al. 1984, Poston 1982).

There are currently no sediment or water quality guidelines for U aimed at protecting aquatic biota. However, Langan (1983) recommended a limit of 300 µg U/L in surface waters for the protection of aquatic life. As discussed in chapter four, concentrations sometimes exceed this concentration in systems impacted by U mining and milling and may approach or exceed acute toxicity benchmarks for some fish species (Appendix four).

U mining and milling activities release numerous metals (Al, Ba, Bi, Bo, Cd, Co, Cr, Cu, Fe, Hg, Mn, Mo, Ni, Pb, V, and Zn), metalloids (As and Se) and a number of radionuclides (U, Th, Ra, Po, and Pb) to the surrounding environment (Hynes 1990). However, little information is available regarding the toxicology of uranium in freshwater

fish. Where adverse effects are observed in biota inhabiting systems receiving U mining and milling wastes, they have generally been attributed to one (or more) of the co-contaminants, with little consideration of the potential role of U (e.g. Anderson et al. 1963). Because of the paucity of data on the toxicology of U in freshwater fish, such conclusions may be invalid.

One of the rare reports of potential effects of U on freshwater fish was generated by evaluations of the Beaverlodge Lake system in northern Saskatchewan, impacted by U mining and milling discharges (Bernstein and Swanson 1989). Packed cell volume, total plasma protein, and red blood cell counts were reduced in lake whitefish and white suckers (*Catostomus commersoni*) from Beaverlodge Lake, relative to fish from the two reference systems. White blood cell counts were higher in suckers from the contaminated system. These differences were attributed to elevated concentrations of radionuclides in the water, sediment, and fish from Beaverlodge Lake.

What remains unclear, however, is the mechanism of these effects, specifically, whether they were manifestations of the chemotoxicity of U or of radiation from the more active radionuclides. While the potential for U to cause these alterations through chemical pathways can not be confirmed, it seems the most plausible explanation. Firstly, radiation doses to these fish were much lower than those shown to adversely affect haematopoietic tissues (Bernstein and Swanson 1989). Secondly, radiation typically causes a reduction in highly radiosensitive white blood cells (Roberts 1978), the opposite of the observed trend. Conversely, metals frequently stimulate an immune system response in fish, thus

augmenting leucocyte numbers (i.e. inflammation) (e.g. Gill and Pant 1985, Sjobeck et al. 1984).

V. Experiment Introduction

Because the diet is the primary route of U exposure to feral fish (Emery et al. 1981, Kovalsky et al. 1967, Swanson 1982, 1983, 1985), data on the acute toxicity of waterborne U are of limited environmental relevance and contribute little to our understanding of the biotic hazards associated with this contaminant. Furthermore, some metals, such as vanadium and selenium, are more toxic to fish when taken up from the diet relative to water (Hilton and Bettger 1988, Hodson and Hilton 1983).

The assessment of the accumulation, distribution, and potential physiological, morphological, and biochemical effects of dietary U in lake whitefish serves to address gaps in our understanding of U toxicology in freshwater fish. The value of this undertaking is threefold. Firstly, it facilitates the identification of the main sites of accumulation and sub-lethal toxic effects of U in a freshwater fish species. Secondly, this study provides insight into the relationships between exposure (and tissue) concentrations and the development of sub-lethal adverse effects. Thirdly, this undertaking elucidates potential candidates as biomarkers that could be applied to environmental monitoring programs. Ultimately, the identification and characterization of dose-related effects and critical concentrations of U in fish is fundamental to the development of environmental guidelines.

The effects of dietary U in lake whitefish were assessed via an evaluation of a suite of biomarkers, chosen in large part from knowledge of U toxicology in mammals, of U exposure and toxicity as has been recommended for assessments of fish health (e.g. Adams et al. 1990, 1996, Depledge et al. 1995, Holdway et al. 1995, Livingstone 1993). Effects were assessed at several levels of biological organization. The general morphometric parameters examined at the whole organism level were: weight, length, condition factor (CF), and growth. At the tissue level, liver somatic indices (LSI), haematological parameters, and histopathology of liver and posterior kidney tissue were evaluated. Finally, biochemical analyses of metallothionein (MT) in liver and kidney, and lipid peroxides (LPO) in serum were investigated. Discussions of the rationales for selecting the biochemical parameters are provided below. The rationale for investigating effects at the tissue and whole organism levels, was to evaluate the relevance of effects observed at the biochemical level, and to assess their utility as bioindicators for use on natural fish populations.

A. Metallothionein

Metallothionein is an ubiquitous, low-molecular-weight (7 kDa), heat stable, cysteine-rich, heat shock protein, whose structure is remarkably similar across taxa (Kille et al. 1992). It has been identified in a number of fish tissues including liver, anterior and posterior kidney, spleen, pancreas, brain, ovaries, testes, heart, gills, and intestines (Chan et al. 1988, Chatterjee and Maiti 1987, Hao et al. 1993, Hylland et al. 1994). Under non-pathological physiological conditions, the primary function of this protein is believed to

be its participation in the maintenance of copper and zinc homeostasis (Olsson 1996), although it has also been implicated in the synaptic events of the brain (Hao et al. 1993). Furthermore, recent mammalian research indicates that MT plays a direct role in cell proliferation in neonates (Wlostowski 1992) and high levels have been associated with various human tumours (Cherian et al. 1994).

MT synthesis is induced by trace amounts of non-essential metals Cd, Hg, and Ag and excessive levels of Cu and Zn in fish (Roesijadi 1992, Wood et al. 1996). In turn, MT sequesters these metals, thus functioning as what is most often interpreted as a detoxification protein. In essence, the chelation of metals is viewed as a defence mechanism because it effectively prevents the free metal ions from interacting with molecular sites and exerting their toxic action (Roesijadi 1992). However, there has been considerable reexamination of this view in the recent literature, as studies emerge reporting that toxic effects of metals may arise despite incomplete saturation of the metal binding sites in the MT pool (Cope et al. 1994). In other words, there is now some question regarding the tendency for metals to bind to the MT that they induce (Blumenthal et al. 1994, Kille et al. 1992). Furthermore, Zn-induced MT synthesis in carp lymphoid cells coincided with depressed non-MT protein synthesis, including antibodies, indicating a possible adverse effect of MT induction upon the teleost immune response (Cenini 1985).

Similarly, Borghesi and Lynes (1996) found that not all of the functions of MT are beneficial as it compromises mammalian immunocompetence, effectively acting as an endotoxin. Furthermore, metal-MT complexes appear to be more toxic in some tissues

(i.e. posterior kidney) than the free metal ions. For instance, Cd is more nephrotoxic as the Cd-MT complex rather than the free metal ion (e.g. Dorian et al. 1995), seemingly paradoxical to the notion of MT as a detoxification mechanism.

The molecular mechanisms by which metals induce MT synthesis have been elucidated in mammals and appear to be similar in fish. In both groups, the MT gene contains metal regulatory elements, two in mammals and four in teleosts, which function as transcriptional promoters (Kille et al. 1995, Kling and Olsson 1995). The induction process occurs via the binding of metals to a transcriptional factor which then experiences a conformational change, enabling it to bind directly to the MT gene. Recently, Kling and Olsson (1995) also identified a potential glucocorticoid responsive element on the teleost MT gene.

The metals Au, Pt, and Bi also induce and bind to MT in mammals, whereas Ni and Co appear to be capable of MT induction but are not chelated by it (Garvey 1990, Petering et al. 1990, Srivastava et al. 1995). In addition, platinum introduced in the form of cis-diamminedichloroplatinum, an anti-cancer drug, induced MT in mammalian tumours concomitant with a development of resistance to the drug and to Cd exposure (Kelley et al. 1988). Comparable MT-inducing abilities of these metals in fish are as yet unknown, although Pb failed to induce MT in cultured turbot fibroblasts (Kille et al. 1992).

More recently, a new protective role has been identified for metallothionein. In addition to its metal sequestering ability, MT scavenges free radicals which may be generated in the presence of metals (Hamer 1986, Matsubara et al. 1987), thus affording

protection to the organism from oxidative stress. For example, in mammals, MT attenuates the adverse effects arising from Ni-induced free radical generation, particularly lipid peroxidation (Srivastava et al. 1995). The potential role of MT as a radical scavenger is further supported by demonstration of its ability to reduce Cd genotoxicity (Coogan et al. 1992) and its ability to inhibit lipid peroxidation (Thomas et al. 1986).

Conversely, there is some evidence that mammalian metal-MT complexes may in fact generate free radicals, which in turn damage DNA *in vitro* (Muller et al. 1991). A similar result was reported in the toxic milk mutant mouse which suffers from a mutation causing excessive Cu-MT in the liver (Stephenson et al. 1994). Cu-MT enhanced the process of lipid peroxidation initiated by an organic hydroperoxide *in vitro*. Overall this suggests that any condition which causes amplification of Cu-MT might result in a predisposition to oxidative stress.

In contrast, few studies have attempted to define the relevance or metabolic cost of contaminant-related MT induction in terms of fish health. A recent examination of these issues revealed that of the biochemical parameters heme oxygenase (HSP30), cytochrome P450 1A, and MT, only the latter, hepatic MT, measured in feral fish failed to correlate to an indicator of fish health, the fish health index (Schlenk et al. 1996). Conversely, laboratory-based studies have demonstrated that MT induction as a result of metal exposure poses a metabolic cost that may manifest as decreased fish growth (Dixon and Sprague 1981).

MT induction in response to metals is manifested as enhanced resistance to further exposure to these same metals, usually measured or defined in fish as increases in median

survival times or LC50's (Couillard and St-Cyr 1997, Klaverkamp et al. 1991). The characteristics of this acclimation response are well defined and similar in fish and mammals. The process begins with an initial lag time associated with the time required for the induced synthesis of *de novo* MT (e.g. Blumenthal et al. 1994, McCarter and Roch 1983). This is followed by an expression of enhanced resistance to numerous metals (Klaverkamp and Duncan 1987, Klaverkamp et al. 1991, Couillard and St-Cyr 1997) in fish and mammals, and to alkylating agents (Kelley et al. 1988) and radiation (Bakka et al. 1982, Matsubara et al. 1987) in mammals; all of which are associated with elevations in MT. In the case of copper, maximal acclimation was achieved in rainbow trout in 7 days (Dixon and Sprague 1981).

The acclimation response is sustained with continued metal exposure as long as a critical threshold of toxicity is not exceeded. Where accumulation of MT-inducible metals exceeds the capacity for MT induction and subsequent metal sequestration, toxicity may be manifested. This characteristic, most commonly referred to as the 'spill-over hypothesis', remains controversial (e.g. Cope et al. 1994, Cosson et al. 1991, Kille et al. 1992). The final feature of a MT stress response is reversibility. Once exposure to the inducing factor is discontinued, resistance also disappears (Chapman 1985, Dixon and Sprague 1981).

Observations of acclimation to U in mammals are similar to those having involvement of MT induction. This feature of U exposure is remarkably similar to MT stress responses observed in mammals and fish exposed to various metals, most notably Cu, Cd, Hg, and Zn. Both U acclimation in mammals and MT induction responses in fish

and mammals display an initial lag time before resistance is manifested, the duration of which is remarkably similar. In mammals exposed to near lethal concentrations of U, the most critical period of toxicity is the first seven to ten days of exposure, when mortality and kidney damage are greatest (Barnett and Metcalf 1949). If mammals survive this initial exposure period, U acclimation is established allowing continued survival to the same concentrations (in the order of years), as well as imparting greater survivability to doses normally toxic to the organism (i.e. without prior exposure) (Haven 1949).

Also like MT-mediated metal acclimation (Chapman 1985, Dixon and Sprague 1981), acclimation to U is not permanent but is instead lost when exposure is discontinued (Haven 1949). Furthermore, the acclimation which develops does not appear to be specific to U. For instance, uranium-induced liver necrosis imparted resistance to the development of hepatotoxicity from both uranium itself and chloroform (Balazs 1974).

U also accumulates in liver and kidney (see chapter two), two tissues in which metal-induced MT synthesis is pronounced. Lastly, U exists predominantly or exclusively as the uranyl ion (UO_2^{2+}) in biological systems (Leggett 1989). Thus it shares a chemical similarity, namely a divalent positive charge, with other MT-inducing metals (Cherian et al. 1994).

B. Lipid Peroxidation

Lipid peroxidation is a process in which the polyunsaturated fatty acids (PUFA) of phospholipid membranes are damaged by free radicals, such as superoxide $(O_2^-\bullet)$ and

hydroxyl (OH•) radicals, and oxygen. The reaction, which is capable of self-propagation, involves the abstraction of a hydrogen atom and terminates in the formation of a lipid hydroperoxide, one of the endpoints of oxidative stress (Di Giulio 1991).

Because basal levels of lipid peroxidation occur in all tissues under normal physiological conditions, organisms are constantly combatting oxidative damage. However, stress which directly or indirectly enhances the production of free radicals or reduces the antioxidant defense systems of a tissue will heighten the process. Lipid peroxidation is biologically relevant as it compromises the structural and functional integrity of biological membranes by increasing membrane structural order and damaging membrane proteins and enzymes (Horton and Fairhurst 1987). Ultimately, lipid peroxidation may cause tissue damage and cell death (Halliwell and Gutteridge 1985, Horton and Fairhurst 1987).

There are two main pathways through which a contaminant may stimulate lipid peroxidation. First, contaminants, particularly metals which exist in more than one oxidation state (Horton and Fairhurst 1987), may enhance free radical production. Metals may function as catalysts in the degradation of lipid hydroperoxides which are normally rather stable. As metabolism of lipid hydroperoxides generates free radicals, expedition of this process heightens lipid peroxidation (Horton and Fairhurst 1987). Contaminants may also increase free radical generation via stimulation of the P450 monooxygenase system (Phase I enzymes) or via direct participation in oxido-reduction reactions and electron transfer pathways (Di Giulio 1991).

The second major route by which lipid peroxidation may be increased by contaminants is via depletion or inhibition of antioxidant defense systems (Di Giulio et al. 1989, Reddy et al. 1981). Several proteins (MT and glutathione) (Di Giulio 1991, Matsubara et al. 1987), vitamins (ascorbate and tocopherol) (Di Giulio 1991), and enzymes (catalase, superoxide dismutase, glutathione peroxidase) (Di Giulio 1991, Di Giulio et al. 1989) function as potent antioxidants due to their ability to sequester and detoxify free radicals. Free radicals may also be generated secondarily to a number of pathologies that may themselves be caused by contaminants. Discussion of these pathways are beyond the present scope and the reader is referred to a recent review by Kehrer (1993) for details.

Metals which increase lipid peroxidation in marine and freshwater fish are Cu, Hg, V, Cd, Pb, and Fe. Increases in the lipid peroxide content of carp liver, gill, and muscle were observed following treatment to waterborne Cu (Radi and Matkovics 1988). Similarly, Cu and Hg increased erythrocyte haemolysis, stimulated superoxide dismutase, and decreased catalase, peroxidase, and glutathione peroxidase activities in the erythrocytes of the marine European sea bass (*Dicentrarchus labrax*) (Gwozdzinski et al. 1992). Vanadium treatment induced hepatic cytochrome P-450 and glutathione-S-transferases in the Indian catfish (*Clarias batrachus* Linn.), likely stimulating lipid peroxidation (Chakraborty et al. 1995). Cd increased lipid peroxidation in rainbow trout (*Oncorhynchus mykiss*) (Palace et al. 1993). Pb caused a dose-dependent increase in lipid peroxidation in the liver of Indian catfish (Chaurasia et al. 1996) and the liver and gills of tilapia (*Oreochromis hornorum*) (Arias et al. 1991). African catfish (*Clarias gariepinus*)

fed Fe-supplemented diets contained elevated malondialdehyde (MDA), a measure of lipid peroxidation, concentrations in liver and heart, but not muscle, and reduced α-tocopherol in liver (Baker et al. 1997).

Specific metals for which lipid peroxidation or free radical generation is an established effect in mammals are: Cd (Reddy et al. 1981), Cn (Ardelt et al. 1989), Cu (Horton and Fairhurst 1987), Fe (Pryor 1966), Hg (Thomas 1990), Ni (Srivastava et al. 1995, Zhuang et al. 1994), Pb (Sifri and Hockstra 1978), Tl (Horton and Fairhurst 1987), and V (Haider and Elfakhri 1991). In addition, Co, Cr, and Ti act as catalysts in the production of free radicals in the laboratory (Pryor 1966).

Many aspects of U toxicology in mammals suggest that adverse effects are mediated by pathways of oxidative stress. For instance, the high affinity of U for mitochondria (Stevens et al. 1980) and its ability to injure this organelle (Carafoli et al. 1971, Leggett 1989) could arise from lipid peroxidation of mitochondrial membranes or disruptions of electron transport (Kehrer 1993). While U-induced acute renal failure does not appear to arise entirely via oxidative stress pathways, the administration of hydroxyl radical scavengers reduced tubular necrosis and the accumulation of BUN and serum creatinine in U-intoxicated rats (Kato et al. 1994). The amelioration of acute renal failure by free radical scavengers suggests that U exerts its toxicity in part via oxidative stress. However, the renal MDA content of these same rats was unaffected by U.

Other lines of evidence supporting the role of oxidative stress and lipid peroxidation in the development of U toxicity are numerous. For instance, approximately 50 years ago it was demonstrated that U treatment stimulated ascorbic acid oxidation in

the kidneys of guinea pigs and that scorbutic animals were more vulnerable to U toxicity (Dounce and Lan 1949). Equipped with contemporary knowledge regarding the biochemistry of oxidative stress, it is conceivable that these observations resulted from stimulation of the process of lipid peroxidation.

Furthermore, additional research conducted as part of the Manhattan Project revealed that acute treatments of U markedly inhibited the catalase activity of mammalian kidney (Dounce and Lan 1949). Because catalase is a potent antioxidant enzyme, a reduced activity could result in, and be caused by, augmented oxidative degradation of this tissue. Conversely, chronically poisoned rats displayed a stimulation of catalase activity in the kidneys (Dounce and Lan 1949). This suggests enhanced oxidative stress in chronically exposed mammals, as stimulation of catalase activity, a common antioxidant response, functions as a detoxification mechanism (Di Giulio et al. 1989).

U also inhibits cytochrome oxidase, the final electron acceptor of the electron transfer system of mitochondria, in mammalian kidneys and liver *in vitro* (Dounce and Lan 1949, Singer et al. 1951). This observation indicates not only that U inhibits mitochondrial respiration, but also suggests a mechanism for free radical generation in the presence of U.

The affinity of U for cellular and subcellular membranes and its ability to damage these structures suggests a potential for U to be participating in processes of oxidative stress. Rat alveolar macrophages incubated in a solution of U exhibited U accumulation in phagosomes (Tasat and de Rey 1987). The U proved cytotoxic and produced notable structural damage, including disruptions of phagosomal membranes and cell death.

However, the most profound result of this study was the demonstration that U is highly toxic to cell membranes. The high avidity of U for membrane phospholipids (Leggett 1989) provides further credence to the possibility that U may enhance lipid peroxidation by behaving as a catalyst in the breakdown of membrane lipid peroxides.

Observations on uranium miners provide further evidence in support of this mechanism of U toxicity. Concentrations of lipid peroxides in the plasma of Czechoslovakian employees of three U mines and in lymphocytes of employees of one mine were increased up to three- and two-fold, respectively, the concentrations measured in reference populations (Sram et al. 1993). However, due to concomitant exposure to other radionuclides and metals, the role of U in the development of this effect is unclear.

The ability of U to inhibit testosterone hydroxylase activities indicates an interaction between U and cytochrome P450 enzymes. Disruption of this enzyme system is a common pathway by which contaminants induce oxidative stress (Di Giulio 1991). U, in the form of nuclear fuel particles, administered by intratracheal instillation, interfered with steroid metabolism in the livers and lungs of rats (Pasanen et al. 1995). Because one of the functions of the microsomal cytochrome P450 enzyme (CYP) superfamily is the hydroxylation of steroids, the activities of testosterone hydroxylases (TxOH) were used as indicators of CYP. Three days post-exposure, T16ßOH activities in liver and lung were depressed in rats treated with neutron-activated (hot) or non-neutron-activated (cold) particles. One and a half years later, testosterone hydroxylation (T16ßOH) activities were markedly lower (reduced up to 70% relative to controls) in the livers of rats treated with 'radioactive' and 'non-radioactive' U. Although no

interpretation of the mechanism(s) by which U produced these effects was given, the similar abilities of hot and cold U to depress testosterone hydroxylation activities suggest that U produced this effect by chemical pathways and not radiation.

The participation of U in the electron transfer pathways of microorganisms suggests the potential for similar behaviours within teleost systems. For instance, microorganisms have been advocated for use in bioremediation of surface waters contaminated by U (Lovley and Phillips 1992) as they reduce hexavalent U in the sediments of aquatic systems via the activities of cytochromes (Lovley et al. 1993). In general, microorganisms use U(VI) as a terminal electron acceptor (Lovley 1993).

The following document is comprised of three chapters and five appendices. Chapters two and three and Appendix one are manuscripts of the data generated from this study. Chapter two describes the accumulation and distribution of dietary U in lake whitefish. In chapter three, the adverse effects of dietary U in lake whitefish are reported. Chapter four is a general discussion of the results and includes an evaluation of the potential radiological hazards of internally deposited U which was accumulated in the lake whitefish used for this research. The final manuscript, presented as Appendix one, is an assembly of base-line parameters of the lake whitefish which serves as a contribution to published databases for an uncommon laboratory surrogate.

Chapter Two

The accumulation and distribution of dietary uranium in lake whitefish (*Coregonus clupeaformis*).

Abstract

The accumulation and distribution of uranium in lake whitefish (*Coregonus clupeaformis*) fed a commercial diet containing 100 µg U/g, 1000 µg U/g, and 10 000 µg U/g for 10, 30, and 100 days were investigated. No food avoidance or refusal occurred. The major sites of U accumulation were the mineralized tissues, bone and scales, intestine, liver, kidney, and, in the highest treatment, gonads. Significant accumulation in fish fed 100 µg U/g was observed only in scales. Duration-dependent accumulation was observed in bone, scales, liver, and kidney, but not in intestine of fish fed 10 000 µg U/g and in scales of fish fed 1000 µg U/g. Dose-dependent accumulation was observed in scales of fish exposed for 100 days. U accumulation in gonads and gill peaked on day 30 when fish gonads were in the most advanced stage of maturation. Analyses of U in intestine, kidney, liver, bone, and scales are recommended for biomonitoring programs designed to evaluate the biological availability of U to fish inhabiting contaminated aquatic systems.

I. Introduction

U is released to the environment as a result of a number of anthropogenic activities, some of which are widespread, such as combustion of fossil fuels (Beck 1989, Burkhart 1991, Cowart and Burnett 1994, Sarosiek et al. 1991) and application of phosphate fertilizers (Bradford et al. 1990, Buhl and Hamilton 1996, Guimond and Hardin 1989), and others which are localized, such as nuclear research facilities and nuclear fuel processing operations (Emery et al. 1981, Hart et al. 1986, Keklak et al. 1994, Rao et al. 1996). Of the known anthroprogenic sources of U to the environment, the most significant contribution arises from U mining and milling (Poston 1982).

Although U enrichment of the environment surrounding U mining and milling operations has been extensively documented (Hynes et al. 1987, Joshi et al. 1989, Kalin 1988, McKee et al. 1987, Swanson 1985, Waite et al. 1989), very little is known regarding the biological availability of U in aquatic ecosystems. U mining and milling is of immediate concern to aquatic ecosystems, because these activities release U into local surface waters (e.g. Hynes 1990), where it may be of concern to the health of resident organisms.

Despite the high solubility of U in surface waters (Cowart and Burnett 1994), the sediments accumulate the highest concentrations and the greatest burden of U in freshwater ecosystems (e.g. Hynes 1990). Concentrations of U found in the surficial sediments of systems impacted by U mining and milling in Canada range from < 100 $\mu g/g$ to > 1000 $\mu g/g$ (dw) (Hynes 1990, Hynes et al. 1987, Joshi et al. 1989, McKee et al. 1987, Swanson 1985). A concentration as high as 5650 $\mu g/g$ (dw) was observed in the

upper two centimetres of sediments of the Wollaston Lake watershed, impacted by the Rabbit Lake U mining and milling operation, in northern Saskatchewan (Neame et al. 1982). The highest concentration reported for Canadian sediment, 18 000 µg/g (dw), occurred in Port Hope Harbour, Lake Ontario, near the site of a U refinery (Hart et al. 1986).

The most significant route of U uptake in freshwater fish residing in impacted systems is ingestion of sediments and contaminated food (Emery et al. 1981, Kovalsky et al. 1967, Swanson 1982, 1983, 1985). Fish species that occupy a benthic trophic position, that feed upon benthic organisms and have a high degree of association with sediments, accumulate the highest concentrations of U, relative to pelagic and predatory species (Emery et al. 1981, Kovalsky et al. 1967, Swanson 1982, 1983, 1985). Of the latter two classes, pelagic feeders accumulate more U than top predators (Rao et al. 1996).

Many field studies have demonstrated that fish are exposed to inorganic contaminants primarily via the diet (Campbell 1994, Everard and Denny 1984, Handy 1996, Harrison et al. 1990, Kashulin and Reshetnikov 1995, Miller et al. 1992). Nonetheless, few studies have evaluated this route of exposure in a controlled laboratory environment. While it is recognized that metals may be more toxic to fish when introduced in the water rather than the diet (Handy 1996), the latter is an environmentally relevant route and may contribute a greater fraction of assimilated metals when fish are simultaneously exposed to both (Dallinger et al. 1987, Handy 1996). This trend has been demonstrated for Cu, Cd, Pb, Zn, and Cd-109 (Harrison and Klaverkamp 1989, Miller et al. 1993, Mount et al. 1994, Vighi 1981). Furthermore, there is evidence that metals

accumulated by fish from their diet are not as rapidly excreted as those taken up from the water (Vighi 1981).

Although contaminant concentrations in sediments of freshwater systems provides valuable information regarding environmental enrichment, and may correlate to metal residues in fish (Bendell Young and Harvey 1989, Johnson 1987), they do not directly relate to contaminant bioavailability to fish (Bradley and Morris 1986). Alternatively, analysis of contaminant concentrations in fish tissues provides more useful integrative information regarding bioavailability and holds more immediate significance in terms of assessing metal-induced stress.

Information regarding accumulation and distribution of U in freshwater fish is limited. Available data, derived from a small number of studies of feral fish, indicate that the primary sites of U accumulation are bone, gastrointestinal (GI) tract, kidney, and liver (Swanson 1985, Waite et al. 1988). No data are available on U accumulation in the tissues of freshwater fish exposed in a controlled laboratory environment, other than rare reports of whole body concentrations achieved via aqueous exposures (Parkhurst et al. 1984, Poston 1982). Measurements of contaminant tissue residues are widely conducted in environmental biomonitoring programs. However, to assess the biological availability of U to fish in contaminated habitats, a thorough study is required of the accumulation and distribution of dietary U.

The present study was undertaken to evaluate relationships between ingestion of U and its accumulation and distribution in freshwater fish tissues. This was accomplished by feeding lake whitefish (*Coregonus clupeaformis*) a commercial diet contaminated with

U at concentrations observed in the sediments of Canadian aquatic ecosystems which have received anthropogenic inputs of U. This evaluation identifies tissues that would serve as the most sensitive and reliable indicators of U bioavailability, and provides useful information on tissues vulnerable to U toxicity. The sub-lethal toxicity of U in lake whitefish is described in chapter three. Lake whitefish was selected because it occupies a benthic trophic level, is of commercial and recreational importance, is relatively sensitive to contaminants, and is ubiquitously distributed and abundant in the Canadian environment, including northern Saskatchewan.

II. Materials and Methods

Fish

Lake whitefish were obtained as eyed eggs from the Dauphin River provincial hatchery near Gypsumville, Man. Fish were hatched and reared, at the Freshwater Institute, Winnipeg, Man. At the commencement of the experiment (December, 1995) fish were 3.5 years of age. Initial wet body weights and fork lengths, expressed as mean ± SE, last measured three weeks prior to the beginning of exposure, were 625 ± 15 g and 34.9 ± 0.3 cm, respectively.

Fish were maintained on a diet of No. 4 trout pellets (Martin Feed Mills, Elmira, Ont.) at a ration equal to 0.8% of body weight given every Monday, Wednesday, and Friday of each week. Fish were allowed a two month acclimation period to all experimental conditions, with the exception of feeding ration which was reduced from

1% of body weight, three weeks prior to the commencement of the experiment. Feeding ration was reduced because at the higher feeding ration, fish did not consume all of the food presented to them. A lower feeding ration ensured that all food was rapidly consumed by the lake whitefish, thereby allowing accurate control of the dose of U administered to fish.

Tanks

Fish were held in rectangular fibreglass tanks with clear plexiglass lids, each tank holding 250 L of water, and each receiving a constant air supply via an airlift system. Tanks received a continuous flow of dechlorinated water (Wagemann et al. 1987) at a rate of 1 L/min, yielding a 90% replacement time of tank volume of 9 hours and a 99% replacement time of 20 hours (Sprague 1973). Tanks were flushed daily to remove wastes accumulated on the bottom of the tank by removing the plunger from the drainage system.

Six fish were randomly allocated to each of 12 tanks. A flow rate of 1 L water/min was sufficient to meet the requirements for limiting ammonia to (0.5 mg/L in each tank according to the methods outlined by Speece (1973). Photoperiod was held constant at 12 hours of light and 12 hours of darkness, with 30 minute periods of 'dusk' and 'dawn'.

Water

Tank water was supplied as dechlorinated City of Winnipeg tap water maintained at or below 8 µg Cl_2/L by activated charcoal filtration and ozonation (Wagemann et al. 1987). Two times during the exposure, this concentration was exceeded due to accidental shutdown of the ozone generators. The first occurred during the acclimation period, 9 days prior to the onset of the experiment. The second, occurred during the experiment on day 95 of the exposure period. On both of these days, chlorine concentrations rose to a maximum of 16 µg/L. In each instance, sodium thiosulfate was added to each tank, approximately one hour after elevated chlorine concentrations were observed, in sufficient quantity to reduce the chlorine to chloride. No mortalities occurred as a result of either event and fish exhibited normal feeding and swimming behaviours.

Tank temperature, pH, and dissolved oxygen concentrations were measured daily and are expressed as mean ● SE. Mean temperature was 10.9 ● 0.1°C and mean pH 7.73 ± 0.01. The mean concentration of dissolved oxygen (DO), 9.6 ± 0.1 mg/L, was 87 ± 1.0% of saturation values. DO within all tanks did not drop below 68% at anytime during the experiment. No significant temperature or pH changes occurred within or between tanks throughout the acclimation and exposure periods.

Major chemistry parameters of water supplying the tanks were analyzed on days 1, 10, and 30 of the experiment (Stainton et al. 1977). The concentrations, expressed as mean ± SE, of major ions were: Ca 24.7 ± 0.3 mg/L, Na 2.32 ± 0.04 mg/L, K 1.50 ± 0.01 mg/L, Mg 6.97 ± 0.03 mg/L, Cl 5.63 ± 0.09 mg/L, and SO_4 3.83 ± 0.07 mg/L. Total anion and cation concentrations were 2011 ± 5 µeq/L and 1946 ± 15 µeq/L, respectively. The concentration of soluble reactive silicon was 1.03 ± 0.01 mg/L. Total dissolved

inorganic carbon and dissolved organic carbon were 1653 ± 15 µm/L and 563 ± 39 µm/L, respectively. Total suspended solids ranged from below the analytical detection limit (DL) of 1 mg/L up to 5 mg/L. Conductivity was 181 ± 1 µS/cm, alkalinity, 1685 ± 23 µeq/L, and the concentration of organic acids, 93.3 ± 12.0 µeq/L.

Nitrites (NO_2), nitrates (NO_3), ammonia (NH_4), and total dissolved nitrogen (TDN) were measured in water supplying the tanks on day one of the experiment and in each of the tanks on each of the three sampling dates. The concentrations of these substances in the incoming water were: NO_2 0.667 ± 0.167 µg/L, NO_3 145 ± 3 µg/L, NH_4 45 ± 5 µg/L, and TDN 467 ± 22 µg/L. Means ($\pm$ SE) of these same parameters within all tanks were: 11.0 ± 2.2, 224 ± 34, 312 ± 29, and 913 ± 63 µg/L, respectively. Thus, the concentration of NH_4 was below the recommended limit of 500 µg/L (Speece 1973).

Exposure

Uranium was obtained as uranyl acetate dihydrate, with an activity of 0.187 µCi/g, from the Fluka Chemical Corporation (Ronkonkoma, NY). The reported isotopic distribution was 99.8% ^{238}U and 0.3% ^{235}U. It contained no reported ^{234}U. To prepare contaminated food, all glassware and labware were acid washed and rinsed with deionized water.

Trout pellets were spiked with aqueous uranyl acetate, to produce nominal U concentrations of 100 µg U/g food, 1000 µg U/g food, and 10 000 µg U/g food. An appropriate amount of uranyl acetate was dissolved in deionized water, transferred to an acid-washed spray bottle, and uniformly sprayed on the trout pellets. Measured

concentrations, expressed as mean ± SE, of U in the fish food were: < 1.25 µg/g in untreated food, 85.5 ± 6.2 µg/g in the low dose, 982 ± 72 in the moderate dose, and 9892 ± 8 µg/g in the high dose.

Each of the 12 tanks was randomly assigned to one of the four dose groups and to one of the three exposure periods. One tank from each dose group was sampled on days 10, 30, and 100 of exposure. Because dose rates to fish were based upon their initial wet body weights (WBW), they decreased with increasing exposure duration due to continued fish growth. These decreases, expressed as percent reduction from initial fish weights, were approximately 4% to 5% at day 10, ranged from 6% to 15% at day 30, and were 15% to 18% at day 100. The exposure regime and dose rates, based upon fish weights at sampling, are presented in Table 2.1.

Food was added to the tanks in several small batches at each feeding. This ensured that the food would be readily consumed upon introduction, thereby reducing the possibility of uranium leaching from the food. This also served to reduce the competition for food within the tanks and, thus, variations in the dose of U received by fish.

Sampling

Four tanks, each containing six fish, with the exception of one tank which contained only five fish, representing each of the four treatment groups were sampled on days 10, 30, and 100 of the exposure period. The tank containing only five fish belonged to the highest treatment group and was sampled on day 30.

Fish were anesthetized with tricaine methane sulfonate (MS-222) (Sigma Chemical Co., St. Louis, MO) at a concentration of 390 mg/L, with pH neutralized by addition of ammonium hydroxide. Fish were removed from the anesthetic after they lost equilibrium and failed to respond to a tail pinch. This occurred in less than three minutes of exposure. Fish lengths and weights were measured and tissue samples were taken as described below.

Removal and Preparation of Tissues

Following blood withdrawal from the caudal area using a heparinized syringe and removal of samples of liver and posterior kidney for histological analyses, tissues were excised and frozen for U and biochemical analyses. The liver was frozen following separation of the gall bladder. The kidney was excised following removal of the swim bladder and overlying mesentery. Lateral incisions were made with a scalpel along the body wall on either edge of the kidney and it was removed with a spatula. The entire gill arches were cut from the fish and frozen. Gill filaments were removed from the gill arches while still frozen, prior to U analysis. The entire GI tract was cut from the abdominal cavity at both its extremities and the contents squeezed out. The intestines were cleaned by making a longitudinal incision, flushing thoroughly with physiological saline, lightly scraping their surfaces, and blotting them dry. The small and large intestine were pooled as a collective sample. Gonads, skeletal muscle, sampled posterior to the opercular bone, scales and the underlying skin from the same region, and both opercular bones were excised. Skin was separated from all overlying scales, and scales and

opercular bone were cleaned of overlying tissues. The latter three tissues were then rinsed in physiological saline and blotted dry. Liver, kidney, and gills were stored frozen at -90°C for both U and biochemical analyses. All other tissues were stored at -20°C for U analysis.

Analysis of Uranium

Tissue and food sample digests were analyzed for uranium using a Spectraspan IIIB Direct Current Argon Plasma Optical Emission Spectrometer (DCP) with ADam software (Spectrametrics Incorporated, Andover, MA). Immediately prior to analysis, all samples were spiked with a solution of cesium chloride, to arrive at a final concentration of 25 μL Cs/mL of sample. Uranium was analyzed at a wavelength of 409.0 nm.

Liver, kidney, gills, intestine (small and large), gonads, skin, scales, bone (opercle), and muscle were analyzed for uranium. A 0.4 to 2.0 g (ww) sample of tissue was weighed in a hot acid washed test tube, and digested in 4 mL of nitric acid and 2 mL of perchloric acid heated at 130°C for 5 hours and at 200°C for 3 hours. The tubes were cooled, 25 mL of deionized water was added, and the samples were mixed by vortex. Two digestive blanks were processed simultaneously to control for contamination.

Trout pellets were analyzed for U to confirm nominal concentrations and to determine background U concentrations in the uncontaminated food. Food was first homogenized using an acid-washed mortar and pestle to aid in its subsequent digestion. Two grams of uncontaminated food and 0.1 g of contaminated food were digested and analyzed for U using the same procedures applied for tissue samples.

Quality Control

There are no certified biological reference materials available for the analysis of U that are suitable for this experiment. Therefore, to verify analytical accuracy, both soft and mineralized tissue samples from unexposed lake whitefish were spiked with known concentrations of U, digested, and analyzed concurrently with tissue samples. These internal reference standards were run regularly (in duplicate every eight samples) and measurements were accepted when the concentration of the internal standards were within 5% of the known concentration of U. The reference samples were prepared by adding U standard to 2 g of muscle and approximately 1 g of bone (opercle) and scales from an unexposed lake whitefish to arrive at a final concentration of 400 µg U/L. The muscle was used as an internal reference for the analysis of all soft tissues. Internal standards of bone and scales were used for the analyses of these mineralized tissues.

Detection Limits

The reported DL of the instrument under the above conditions is 80 µg U/L (Spectrametrics Incorporated, Andover, MA). An actual DL was determined by repeated analysis of samples of muscle from an unexposed lake whitefish spiked with a known range of U concentrations and by analysis of increasingly lower concentrations of uranium standards. The actual DL was determined to be 100 µg U/L. Because the weight of tissue used for the U analysis varied, DL were tissue-dependent. The DL, reported as wet weights, for the nine tissues were as follows: bone 6.25 µg/g, gill 1.67 µg/g, testis

2.50 µg/g, ovaries 1.25 µg/g, intestine 3.57 µg/g, kidney 3.13 µg/g, liver 2.08 µg/g, muscle 1.25 µg/g, scales 1.43 µg/g, and skin 1.25 µg/g. The DL for fish food was 1.25 µg/g.

Statistical Analysis

Where U was not detected, a concentration equivalent to one half of the DL was assigned. This assignation was made under the assumption that concentrations of U below the tissue DL were normally distributed with a mean lying in the centre of this distribution curve. Concentrations of U in lake whitefish tissues were analyzed by a one way analysis of variance (ANOVA) using the SigmaStat 2.0 (Jandel Scientific, Corte Madera, CA) Software Package within each block of time and within each treatment. As the assumptions of normality and equal variance necessary for application of a One Way ANOVA were not met, analyses were performed using the Kruskal-Wallis One Way ANOVA on Ranks. Where differences were significant, treatment group means were compared to controls using the Dunnett's or Dunn's multiple comparison methods. The concentrations of U in tissues of the two groups of lake whitefish fed similar cumulative total quantities of U, were analyzed by the Student's t-test. Fish growth data were analyzed by a One Way ANOVA. Significance was tested in all methods at $\alpha = 0.05$. Linear regression analyses were performed on data for determining the strength of dose- and exposure duration-dependent accumulation (95% C.I.; $\alpha = 0.05$). All data are presented as mean ± SE, unless otherwise indicated. Tissue residues are reported as wet weights.

III. Results

Feeding and Fish Growth

The growth of fish fed contaminated diets did not differ significantly from growth in the respective control groups (Figure 2.1). All, but one (see below), fish consumed food presented in all four dose groups throughout the 100 days of exposure. The GI tracts of all sacrificed fish contained food. Addition of food to the tank had no effect on tank water chemical parameters. Uranium was not detected in tank water, even following addition of contaminated food.

With one exception, no overt signs of toxicity, including behavioural alterations, were observed throughout the exposure period. One female fed the highest concentration of U exhibited impaired buoyancy control and appeared lethargic beginning on day five of the exposure period. By day eight, this individual displayed a complete loss of buoyancy control, scale loss, hyperventilation, a failure to eat, and was floating on its back. On day 10, when fish in this tank were sacrificed, the condition of this female had deteriorated even further, to a "nearly moribund" state.

Evidence of Gross Pathology

On the first sampling day (d 10), internal gross pathologies occurred in fish from the highest treatment group only. Of the six fish fed the highest treatment diet sampled on

day 10, one male and one female had spotted livers. The liver of the nearly moribund female was severely mottled white, with pronounced white spots on its dorsal surface, and the gallbladder contained black bile.

By sampling day 30, overt pathologies were evident in fish from the moderate and high exposure groups but not in fish from the control and the lowest treatment groups. Two fish fed 1000 µg U/g food for 30 days exhibited mottled livers, one of which was mostly white in coloration. Two of the six fish receiving the diet with the highest U concentration had discoloured and spotty livers on day 30. The ovaries of most fish from all exposure groups sampled on day 30 (including controls) were in an advanced state of maturation.

By day 100, fish from all three treatment groups displayed some gross pathologies. The control group remained without overt pathologies by day 100. Gonads in two of the three females fed 100 µg U/g food, and in all three females fed 1000 µg U/g food, had begun to redevelop by day 100. One of the two females fed 10 000 µg U/g food that were sampled on day 100 had developing gonads. An external lesion, described as a skin ulceration, was present near the tail of one male fish fed the highest concentration of U. All fish consuming contaminated diets had mottled livers on day 100.

Accumulation of Uranium

Figure 2.2 presents the concentrations of U achieved in nine tissues of lake whitefish fed an uncontaminated diet and diets containing three concentrations of U for 10, 30, and 100 days. U was not detected in the tissues of unexposed fish. Concentrations

of U in the tissues of fish from the 100 µg U/g group, were, with one exception, below analytical detection limits and were not significantly greater than controls. Significant accumulation of U was observed in the scales of lake whitefish exposed to 100 µg U/g food for 100 days. In fish fed the moderate concentration of U, 1000 µg U/g, significant accumulation occurred in bone, scales, kidney, and intestine. Significant accumulation of U occurred in all tissues, except muscle and skin, of lake whitefish fed 10 000 µg U/g on at least one sampling day. One outlying point (594 µg/g) was discarded for kidney in the group fed 10 000 µg U/g food for 10 days. It was assumed that this unusually high value was a result of sample contamination and was therefore not included in the statistical analysis or in the presentation of results in Figure 2.2.

The distribution of U in nine tissues of lake whitefish fed 10 000 µg U/g food is presented in Figure 2.3. The intestines contained U at concentrations one to two orders of magnitude greater than all other tissues. The next highest concentrations were located in bone and scales. Liver, kidney, and gonads also proved to be major sites of accumulation.

Groups of fish fed similar total amounts of U, accumulated similar concentrations. For example, fish fed 10 000 µg U/g food for 10 days and fish fed 1000 µg U/g food for 100 days had ingested a cumulative total of 307 µg U/g fish and 284 µg U/g fish, respectively. Concentrations of U in fish from these two treatment groups were similar in all tissues but intestine (Figure 2.4).

Accumulation of U was duration-dependent in bone ($R^2 = 0.9146$, $P < 0.0001$), kidney ($R^2 = 0.5552$, $P < 0.001$), scales ($R^2 = 0.9258$, $P < 0.0001$), and liver ($R^2 = 0.7586$, $P < 0.0001$) of fish from the highest treatment group (Figure 2.5), and in scales ($R^2 =$

0.5124, P < 0.001) of fish from the moderate treatment group (Figure 2.6). Accumulation in gills and gonads was highest on day 30, when gonad growth was most marked. Although concentrations in these tissues were significantly higher on days 30 and 100, relative to those measured on day 10, no clear duration-dependent accumulation was apparent. Because little significant accumulation of U occurred in the tissues of fish from the low and moderate exposure groups, or insufficient data points were obtained due to detection limit restrictions, linear regression analysis of duration-dependent accumulation could not be performed on these groups, with the one exception (scales) noted above.

Strong dose-dependent accumulation of U was observed in scales of treated fish sampled on day 100, using log-transformed data (R^2 = 0.8916, P < 0.001) (Figure 2.7). Because fish from the lowest treatment group did not accumulate U in any tissue but scales, no other tissue could be evaluated for dose-dependent accumulation by linear regression analysis.

A highly significant positive correlation (R^2 = 0.8734, P < 0.0001) was also demonstrated between concentrations of U in fish scales and the total mass of U ingested by fish (Figure 2.8). Only those groups where significant accumulation occurred in this tissue were included in this regression analysis (i.e. all treatment groups except the low treatment on days 10 and 30).

IV. Discussion

This study was conducted to evaluate the potential for a freshwater fish to accumulate U introduced in the diet and to identify the main tissues where U is

accumulated. The lake whitefish in this study readily consumed concentrations of U as high as 10 000 µg U/g food with no evidence of adverse taste imparted to the food by the addition of uranyl acetate. Because U was added to the surface of the feed, rather than by direct incorporation, this exposure should represent a 'worst case scenario' in terms of evaluating food avoidance reactions. Fish growth data, in conjunction with direct observations of continued feeding and the presence of food in the GI tracts of all sacrificed fish, support the assumption that the theoretical dose of U administered to fish was achieved.

Intestine

Substantial accumulation of U occurred in the intestines of lake whitefish fed 1000 µg U/g and 10 000 µg U/g. The intestines of fish from the highest exposure group contained U at concentrations one to two orders of magnitude greater, the highest of all tissues, than fish fed the low and moderate test diets. The mean concentration of U (879 ± 369 µg/g), observed in the intestines of fish from the highest exposure group sampled on day 100, was 63 times greater than in fish from the moderate exposure group (14.0 ● 2.3 µg/g). However, accumulation in this tissue was not duration-dependent in fish fed 10 000 µg U/g food, despite slight increases in mean concentrations over time. This may indicate that an equilibrium concentration had been reached in this tissue within 100 days of exposure.

Analyses of feral fish have also revealed that fish GI tracts accumulate U. Fish inhabiting Beaverlodge Lake, Saskatchewan, a system contaminated by U mining and

milling discharges, consistently contained the highest concentrations of U in the stomach (Swanson 1985). Conversely, lake whitefish sampled in Langley Bay, Lake Athabasca, Saskatchewan, contaminated by tailings deposited by the Gunnar Uranium Mine, contained the highest concentration of U in bone, followed by kidney (Waite et al. 1988). Although detected, the U concentration in gut, 0.1 µg U/g (ww), was six times lower than that observed in kidney and only twice as high as that found in male gonads and muscle (Waite et al. 1988). It appears that at low exposure concentrations, such as those encountered in Langley Bay and in the group of lake whitefish fed 100 µg/g in this study, accumulation in intestines is disproportionately lower, relative to other tissues, than when fish are exposed to higher U concentrations.

A number of other metals have been found to accumulate largely in the GI tracts of fish when exposure is achieved mainly or exclusively via the diet (Farag et al. 1995, Haines and Brumbaugh 1994, Handy 1992, Harrison et al. 1990, Miller et al. 1992, Pelletier and Audet 1995). High concentrations of metals in gut tissues relative to gill, as was demonstrated in this study, are indicative of the route of exposure (Dallinger et al. 1987).

Bone & Scales

High concentrations of U were achieved in the highly mineralized tissues, bone and scales, of exposed lake whitefish. Accumulation in both tissues was highly dependent upon exposure concentration and duration. U's affinity for fish bone has been demonstrated in feral lake whitefish (Swanson 1982, 1983, 1985, Waite et al. 1988),

white sucker (*Catostomus commersoni*) (Bernstein and Swanson 1989, Swanson 1982, 1983, 1985), lake trout (*Salvelinus namaycush*) (Swanson 1983), brown trout (*Salmo trutta*) (Pettersson et al. 1988), and arctic char (*Salmo alpinus*) (Pettersson et al. 1988) residing in habitats contaminated by U mining and milling wastes, as well as in fish inhabiting naturally enriched systems (Kovalsky et al. 1967, Mahon 1982). U is considered a bone-seeker in mammals due to its high affinity for calcium-rich structures (e.g. skeleton) and its high retention in this tissue (Leggett 1994).

Correlations between U in fish bone and abiotic concentrations, as was observed here, have been reported for natural populations. Evaluations of the radionuclide content of fish inhabiting systems in the Lilljuthatten area, northern Sweden, revealed a relationship between U in skeletal tissues and an environmental gradient (Pettersson et al. 1988). Concentrations of ^{238}U and ^{234}U in the bone of arctic char and brown trout decreased with increasing distance downstream of a U mineralization, that had been subject to prospecting.

U also displayed an affinity for fish scales, particularly evident in lake whitefish fed the highest U concentration. Furthermore, scales are the only tissue in which significant accumulation of U occurred in the lowest treatment group. Accumulation in lake whitefish scales proved to be both highly dependent upon exposure dose on day 100 and duration in the high and moderate treatment groups. As for bone, no evidence of an accumulation plateau was seen in this tissue within 100 days. In fact, accumulation of U in scales was shown to be a linear function of the total mass of U ingested by lake whitefish. This relationship further indicates that U concentrations in scales of feral fish

would be good predictors of environmental exposure. To the authors knowledge, no data are available on U accumulation in the scales of fish exposed in the laboratory or in the natural environment.

U displayed a strong affinity for both bone and scales; tissues which share some basic structural similarities. Both contain a collagenous matrix in which minerals are deposited and a mineralized crystal lattice composed of the same basic building block, hydroxyapatite. Early research on the accumulation of U in freshwater fish revealed that U, in the form of the uranyl ion, displaces Ca from the skeletal hydroxyapatite crystal lattice, thus functioning as a Ca antagonist (Kovalsky et al. 1967), as it does in mammals (Neuman 1953, Rowland and Farnham 1969). Although the present data do not address the molecular site (i.e. in the highly calcified osseous layer or the lower collagenous fibrillary plate) of U deposition in lake whitefish scales, it is reasonable to speculate that a behaviour similar to that observed in bone is occurring in this tissue.

A number of other metals and radionuclides accumulate in the mineral matrices of fish scales (Moreau et al. 1983, Pender and Griffin 1996). ^{90}Sr, an element which shares a mammalian biokinetic model similar to that applied to U (Leggett 1994), accumulates primarily in the bone and scales of the common carp (*Cyprinus carpio*) (Stanek et al. 1990). Numerous other metals, including Zn, Mn, Pb, Co, and Sr, accumulate in the scales of fish exposed in the laboratory (Sauer and Watabe 1984, 1989).

The mechanism of metal accumulation in fish scales may involve the release of metals from ligands as a result of the slight drop in pH encountered in regions of active calcification. This was suggested for Zn, because it is deposited in actively growing scale

circuli (Sauer and Watabe 1984, 1985). This mechanism also seems plausible for U because carbonate ions are principle carriers of U in the circulation and metal-carbonate complexes become increasingly unstable with decreasing pH (Stevens et al. 1980). Furthermore, the alkaline earth elements Pb and Sr, which behave similarly to U in mammalian bone (Leggett 1994), are deposited in the mineralized regions of fish scales (Sauer and Watabe 1984, 1989).

Kidney

Lake whitefish kidney proved to be a main site of U accumulation, in agreement with the literature. Kidney of lake whitefish from Langley Bay, Saskatchewan contained the second greatest concentration of U, exceeded only by bone (Waite et al. 1988). Furthermore, the mean concentration in kidney was three fold higher than liver and ovaries, six times higher than gut, and an order of magnitude greater than male gonads and muscle. A similar relative tissue distribution, where concentrations of U in kidneys were second only to those in bone, was found in fish inhabiting the naturally enriched Lake Issyk-kul, Russia (Kovalsky et al. 1967). Although kidneys contained only 0.04% of the total body burden, concentrations were 10 to 60 times higher than in kidneys of fish from a reference lake.

Although the mechanism of U accumulation in the kidney of lake whitefish was not investigated, it may resemble a mechanism identified in mammals. In mammals, including humans, kidney is one of the main sites of accumulation, and the critical target tissue for the chemotoxic action of U (Leggett 1989, 1994, Singh et al. 1987a, Tracy et al.

1992, Voegtlin and Hodge 1949, 1953, Wrenn et al. 1985). The mechanism of deposition in mammalian kidney involves ultrafiltration of uranyl-carbonate complexes, one of the predominant complexes in the circulation (Stevens et al. 1980), across glomeruli. The complex dissociates in the acidic environment of proximal tubule lumens and U binds to tubular brush borders where it may be taken up into the epithelium (Dounce 1949).

Liver

Significant, and strong duration-dependent, accumulation of U occurred in the liver of lake whitefish fed the highest concentration of U. Other studies have reported elevated concentrations of U in the livers of fish inhabiting contaminated environments as well as correlations to environmental concentrations. For example, during periods of decreased water flow (i.e. July), hepatic concentrations of U, although relatively low, in rainbow trout (*Salmo gairdneri*) collected at intervals downstream of a seepage from a U mine wastepile in Washington, decreased with increasing distance along the environmental gradient (Nichols and Scholz 1989).

Significant accumulation in the livers of fish fed low and moderate concentrations of U were not observed in this study. If the results obtained from the highest treatment group are representative of longer term exposures to lower concentrations of U, then it is anticipated that U would continue to accumulate in this tissue over time. Results of field studies imply that under chronic exposure regimes, liver is a significant site of U accumulation in fish. Lake whitefish and white sucker from Beaverlodge Lake contained a mean (± SE) concentration of U of 3.76 ± 0.87 µg/g (ww) and 2.07 ± 0.44 µg/g (w.w.)

in the liver, respectively (Swanson 1985). Lake whitefish from Langley Bay, contained low, but detectable concentrations of U in the liver (0.2 μg/g ww) (Waite et al. 1988). These concentrations are remarkably close to, and in some instances lower than, the present detection limits of 2.08 μg/g and may explain the failure to find significant accumulation in the livers of fish from the moderate and low treatment groups.

Gonads

Only fish fed 10 000 μg U/g food accumulated significant concentrations of U in gonads. Mean concentrations were significantly elevated above controls on all three sampling days, however no clear relationship between exposure duration and accumulation was evident. Although U concentrations in gonads of fish sampled on day 30 and 100 were not significantly different, the mean concentration of U was highest on day 30.

In fish fed the intermediate concentration of 1000 μg U/g, U was detected in one fish sampled on day 10, one on day 100, and in four fish sampled on day 30. Although not statistically significant, the maximum concentration observed in the gonads, and the frequency of detecting U in fish gonads, were greatest on day 30 in fish fed 1000 μg U/g. U was not detected in the gonads of fish fed the lowest concentration of U.

There are several possible explanations for the occurrence of maximal accumulation of U in gonads on day 30. The first relates to U dosage, which was duration-dependent. Fish continued to grow throughout the exposure period, yet the concentration of U in the food and the feeding ration remained constant. Feeding ration

was not adjusted over the exposure period because of the stress imposed upon fish due to handling and anesthetization when obtaining fish weights. Therefore, there was a progressive decline in the dose of U administered to fish with increasing time; a reduction which may have contributed to decreased accumulation in gonads on day 100. However, if this explanation were adequate, it would be anticipated that maximal accumulation of U in other tissues would coincide with that observed in gonads (i.e. on day 30). As this was observed for only one other tissue, gill, this explanation is insufficient.

A second explanation relates to gonadal maturation. The ovaries of most females sampled on day 30 were at the most advanced state of maturation of the three sampling days. Although spawning did not occur, it is probable that the metabolic activities of male gonads were also heightened, synchronous with ovarian growth; a reproductive concurrence typical of salmonids (Scott 1990). Because of the increased metabolism which accompanies the sexual maturation of gonads and subsequent new tissue growth, this process may have heightened the delivery and, subsequently, accumulation of U in the ovaries. If the metabolic activity of testes were also heightened, a similar argument could be made for augmented accumulation in males. Although sample sizes were inadequate for statistical evaluation, testes accumulated higher concentrations of U than ovaries. The mean concentration of U in the three males belonging to the highest treatment group sampled on day 30 was 194 ± 50 μg/g (ww). This is approximately an order of magnitude greater than the mean concentration (17.8 ± 3.4 μg/g ww) achieved in the ovaries of the two females from the same group.

This apparent sex-related difference may be explained by a tissue dilution effect in female gonads that is similar to that proposed to account for decreased accumulation of metals in larger relative to smaller fish. Ovaries which have grown substantially may contain lower concentrations of U than the considerably smaller testes, even when the tissue burdens are equivalent. This 'growth dilution' in ovaries may occur despite increased U delivery by the circulation.

Another postulation which could explain the non-linear accumulation of U in fish gonads from this study, was generated by research into the behaviour of U in aquatic systems thirty years ago. Fish (species unreported) residing in Lake Issyk-kul, Russia, contained elevated concentrations of U in hard and soft tissues (Kovalsky et al. 1967). Accumulation of U in gonads was reported to be 'relatively independent' of environmental concentrations. The authors suggested that U participates in the metabolic processes of fish gonads, where U was perceived as 'playing a functional part in fish reproduction'. Furthermore, U was directly incorporated into the cellular structures of fish gonads, showing a particular affinity for nuclei and mitochondria (Kovalsky et al. 1967). These results indicate that U is maternally transferred into ova, and that the concentration of U achieved in the ovaries is dependent upon the stage of gametogenesis. The results of this study are in agreement with the latter point. Whether U plays a pivotal role in the processes of fish gametogenesis and reproduction has not been addressed.

A likely explanation for gonadal maturation in the lake whitefish used in this study is the persistence of endogenous rhythms. Fish held in a controlled environment may sexually mature independently of environmental variables (Sumpter 1990) and it has

been reported that there is sufficient evidence for an endogenous obligatory reproductive cycle in salmonids (Scott 1990, Sumpter 1990). The timing of gonadal development in the experimental lake whitefish corresponded to spawning times of natural populations. The experiment was conducted over the winter and feral lake whitefish in Canada spawn in the fall or early winter (Scott and Crossman 1973).

Other Soft Tissues

The accumulation of U in the remaining soft tissues analyzed was negligible, relative to those tissues discussed above. Although U was detected in muscle and skin, no significant accumulation occurred in these tissues in any of the three treatment groups. Muscle consistently contained the lowest concentrations of U. The maximum concentration, 3.51 μg/g, occurred in an individual fed 10 000 μg U/g for 10 days. There was no evidence of duration-dependent accumulation in this tissue. The highest concentration observed in skin, 5.35 μg/g, occurred in an individual fed 10 000 μg U/g food for 30 days.

The pattern of accumulation in gill filaments was similar to that observed in gonads. Significant, but non-duration-dependent, accumulation was demonstrated only in the highest treatment group on days 30 and 100. As was observed in gonads, the highest concentration in gill occurred on day 30. This peak may be a consequence of enhanced oxygen and nutrient demands accompanying concomitant gonadal development. Stimulation of gill metabolism could result in increased blood supply, and likewise, U delivery.

Alternatively, the maximal accumulation of U in gill observed on day 30 may be a reflection of stress arising from both the simultaneous presence of U and the demands associated with gonadal growth. U-induced stress may be compounded by the metabolic demands of sexual maturation, and ultimately could cause an increase in oxygen demand when the two coincided on day 30. It is conceivable that under non-spawning conditions, fish are able to cope with stress imposed by U, but under the added demands associated with gonadal maturation, the capacity of adaptive mechanisms may be overwhelmed and secondary stress effects may emerge.

Other evidence, in particular elevations in serum chloride concentrations, also observed on day 30 (see chapter three), supports the hypothesis of heightened metabolic activity in these fish. Metabolic acidosis, indicated by an increase in serum chloride, may cause a decline in the oxygen content of blood, in particular by decreasing the affinity of O_2 for haemoglobin (Barton and Iwama 1991). Ultimately, an endogenous hypoxic condition is created which is counteracted by stimulation of gas transfer at the gill surface, mediated by secretion of epinephrine from chromaffin tissues (Barton and Iwama 1991). This stimulation of respiratory activities could account for heightened U accumulation in gill.

Accumulation as a Function of Total Ingested U

Lake whitefish that had consumed a similar total mass of U accumulated similar concentrations in eight of the nine tissues examined. This indicates that U continued to accumulate in lake whitefish fed contaminated diets independently of exposure

concentration and duration, for up to 100 days. The one tissue exempt from this trend is intestine. Concentrations of U in the intestines of fish from the highest treatment group were 34 times higher than in those of fish from the moderate treatment group. It should be noted that a great deal of caution was exercised in rinsing and removing U and U-containing food and faecal particles from the intestinal epithelium prior to analysis. Thus, it is unlikely that the high concentrations observed in this tissue are attributable to either surface adsorption or sample contamination.

No immediate explanation is evident for the discrepancy between U accumulation in the intestines of fish from these two groups. Possibilities include differences in retention times and/or accumulation mechanisms associated with varying dosage. The former explanation has been supported by mammalian data. Retention of U in mammalian kidney may be greater at high exposure doses than at lower doses (Leggett 1994, Jones 1966). In addition, high concentrations of U in the diet may induce the synthesis of metal-binding proteins in intestines, ligands which could subsequently augment accumulation and retention in this tissue.

The high concentration of U in the intestines of fish fed high dietary concentrations, are indicative of the existence, and saturation, of an intestinal active transport mechanism. If U was transferred from the gut to the blood by simple diffusion processes, the relative concentrations of U in the nine tissues evaluated should have remained constant under both exposure regimes. Disproportionately high concentrations in the intestine could result from saturation of an active transport pathway. Further research into this postulation is required.

Alternatively, excessive accumulation of U in the intestines of fish fed the highest concentration of U may be a result of structural damage to this tissue, a possibility that requires further investigation. High concentrations of U, like other metals, in food may overwhelm non-specific protective functions of fish GI tracts, which retard passive absorption from the gastrointestinal lumen, and may cause cellular damage resulting in increased accumulation. For example, chronic ingestion of a diet contaminated with Hg caused structural damage in the gut of rainbow trout (*Oncorhynchus mykiss*) which, in turn, resulted in elevated accumulation of Hg by diffusion processes (Handy 1996).

V. Summary and Recommendations

The tissues that were the most sensitive and reliable indicators of U bioavailability in lake whitefish were bone, scales, intestine, liver, and kidney. As osseous tissues accumulated high concentrations of U, scales and bone would be the most sensitive indicators of environmental U exposure. Analysis of intestine is recommended as it may be a significant site of accumulation and because it provides information regarding the route of exposure (Dallinger et al. 1987). Liver and kidney are important bioindicator tissues because both are sites of accumulation and both provide biologically relevant information as they are vulnerable to U toxicity (see chapter three). Although the accumulation of U in lake whitefish gonads was irregular, this tissue should also be considered in biomonitoring programs of contaminated environments because of the potential for a high degree of accumulation, possibly greatest during the reproductive period.

There remain a number of issues to be resolved regarding accumulation of U orally administered to fish. Because the present study does not provide empirical evidence that U accumulation in fish gonads is greatest during the reproductive cycle, this area should be thoroughly addressed. Furthermore, additional research is required to substantiate sex-related disparities in U accumulation observed in the present study. The potential effects of U on fish gonads, development, and maturation and on reproduction should also be examined in light of the high concentrations achieved in this tissue. Furthermore, limited evidence generated by research conducted as part of the Manhattan Project (1942-1945), indicates that U may be a potent reproductive toxin in mammals (Maynard and Hodge 1949, Maynard et al. 1953). However, little attention has been paid to these early observations and there remains a paucity of data on this subject (Stopps and Todd 1982). The ability of U to impair teleost reproduction requires similar attention.

It would also be relevant to determine the maternal transfer potential of U in freshwater fish eggs. The molecular site(s) of U accumulation in lake whitefish bone and scales has yet to be demonstrated and is of significance in determining the potential for adverse effects in these tissues as well as the potential for depuration and remobilization. Ultimately this information would help to gauge the reliability of these tissues as long-term indicators of U exposure in fish. U is mobilized from mammalian bones in the short-term, by cation exchange at the bone surface, and in the long-term, through bone resorption (Leggett 1994). Other metals, such as Zn, which are deposited in the mineral matrices of fish scales may also be mobilized, possibly via scale resorption (Sauer and

Watabe 1989). Remobilization of U from bone and/or scales could serve as a source of U to the soft tissues after exposure is discontinued.

As this study did not attempt to address the mechanisms of accumulation and the kinetics of depuration, these areas also remain to be explored. It would be of use to investigate the mechanisms of U uptake across the gut and its deposition in the various tissues. Estimation of the fractional uptake of dietary U (i.e. GI transfer coefficient), which has been the focus of mammalian researchers for many years (Leggett 1994), in teleosts would have immediate applications. This coefficient would be very effective in modelling exposure in natural environments and subsequently aid in establishing guidelines for the protection of aquatic life. It would also be of use to evaluate U accumulation at lower exposure concentrations under longer durations.

Finally, analyses of U in the scale annuli of fish inhabiting contaminated environments could provide a chronological record of U exposure and could be compared to concentrations in the strata of sediments from the same system, measurements which provide information on the temporal accumulation of U in the abiotic environment. As the scales appear to be major sites of U deposition and could potentially be collected by non-invasive techniques, they are very promising candidates for biomonitoring programs designed to assess the bioavailability of U to fish.

Table 2.1. Exposure regime and U dose rate to lake whitefish fed uncontaminated and contaminated diets for 10, 30, and 100 days. Nominal and measured [U] in food are expressed as mean (± SE). Daily dose rates were calculated using both initial (pre-exposure) and final (at sampling) wet body weights (WBW). The reduction in dose due to fish growth is expressed as a percentage decrease from the initial (d 0) exposure dose.

Day	Treatment Group	Nominal [U] In Food (µg U/g)	Measured [U] In Food (µg U/g)	Calculated Daily U Dose to Fish[a] (µg U/g fish/d)		Reduction in Dose From Day 0 Due To Fish Growth (% decrease from initial dose)	Calculated Cumulative Total of Ingested U[a] (µg U/g fish)	
				Using Initial WBW	Using WBW at Sampling		Using Initial WBW	Using WBW at Sampling
	Control	0	0.625 (0.000)	-	-	-	-	-
10	Low	100	85.5 (6.22)	0.320	0.305	4.83	3.20	3.05
	Moderate	1000	982 (71.7)	3.20	3.05	4.84	32.0	30.5
	High	10000	9892 (754)	32.0	30.7	4.01	320	307
30	Low	100	85.5 (6.22)	0.347	0.296	14.7	10.4	8.87
	Moderate	1000	982 (71.7)	3.47	3.25	6.38	104	97.4
	High	10000	9892 (754)	34.7	31.5	9.04	1040	946
100	Low	100	85.5 (6.22)	0.344	0.291	15.4	34.4	29.1
	Moderate	1000	982 (71.7)	3.44	2.84	17.4	344	284
	High	10000	9892 (754)	34.4	28.3	17.8	3440	2827

[a] Calculations of daily dosages and cumulative totals of ingested U were made as follows:

(1) Mass of food consumed/fish: Total Tank WBW$_{Initial\ or\ At\ Sampling}$ (g) * Feeding Ration (0.008 g food/g fish/feeding) * Number of Feedings / Number of Fish Per Tank = A (g food consumed/fish)

(2) Cumulative total of ingested U: A (g food consumed/fish) * [U]$_{Food}$ (µg U/g food) / Tank Mean Fish WBW$_{Initial\ or\ At\ Sampling}$ (g) = B (µg U/g fish)

(3) Daily U dose to fish: B (µg U/g fish) / Number of Days of Exposure (days) = C (µg U/g fish/d)

Figure 2.1. The growth of lake whitefish fed uncontaminated and U-contaminated diets for 10, 30, and 100 days. Data, expressed as mean ($\pm$ SE), are presented as a percentage increase from initial wet body weights.
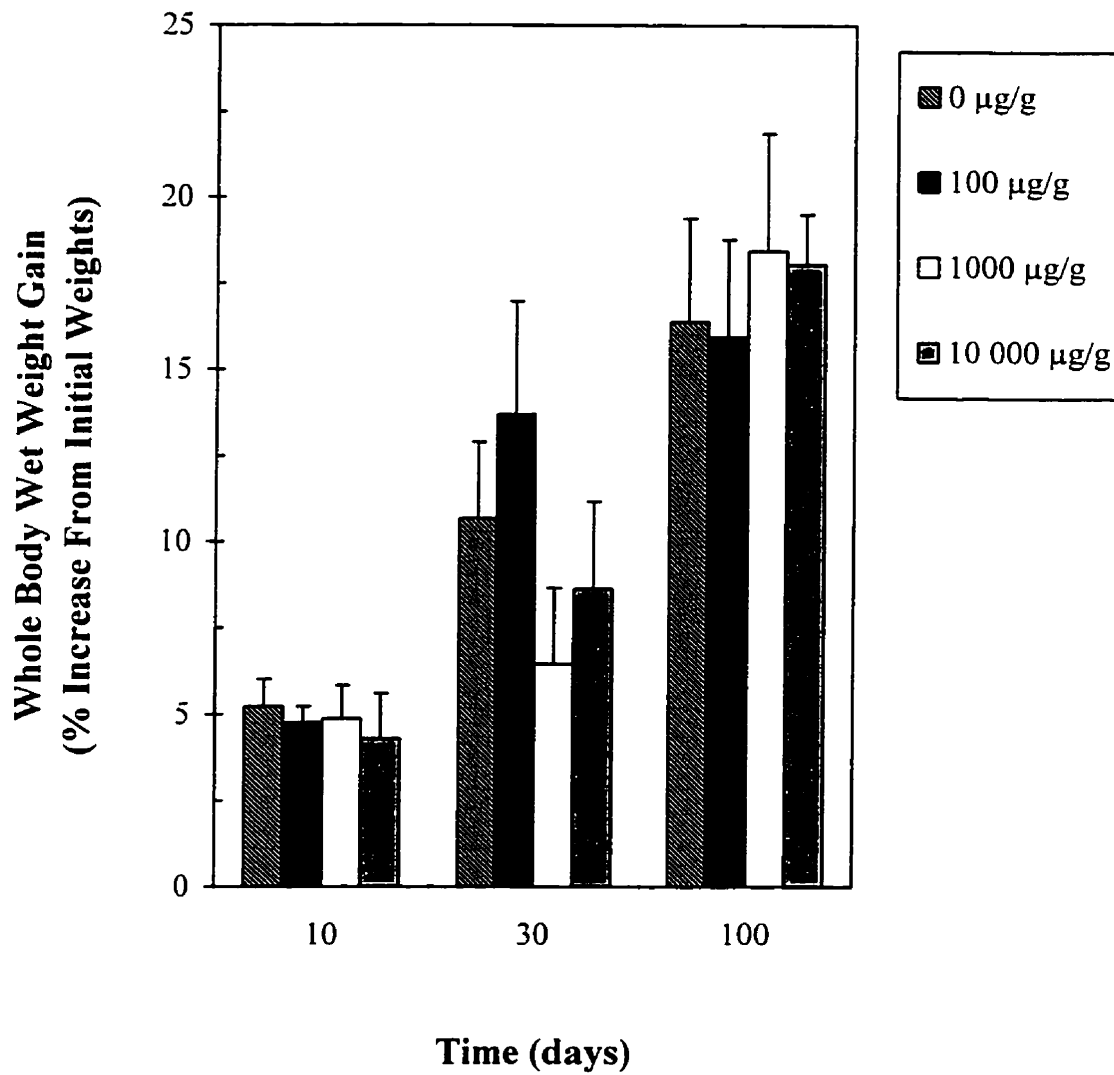
Figure 2.2. The accumulation of U in nine tissues of lake whitefish fed an uncontaminated diet and diets contaminated with U for 10, 30, and 100 days. (A) bone, (B) gill, (C) gonad (D) intestine, (E) kidney, (F) liver, (G) muscle, (H) scales, and (I) skin. Data are expressed as mean ($\pm$ SE). Asterisks denote means significantly different from controls (P < 0.05).

(A) Bone
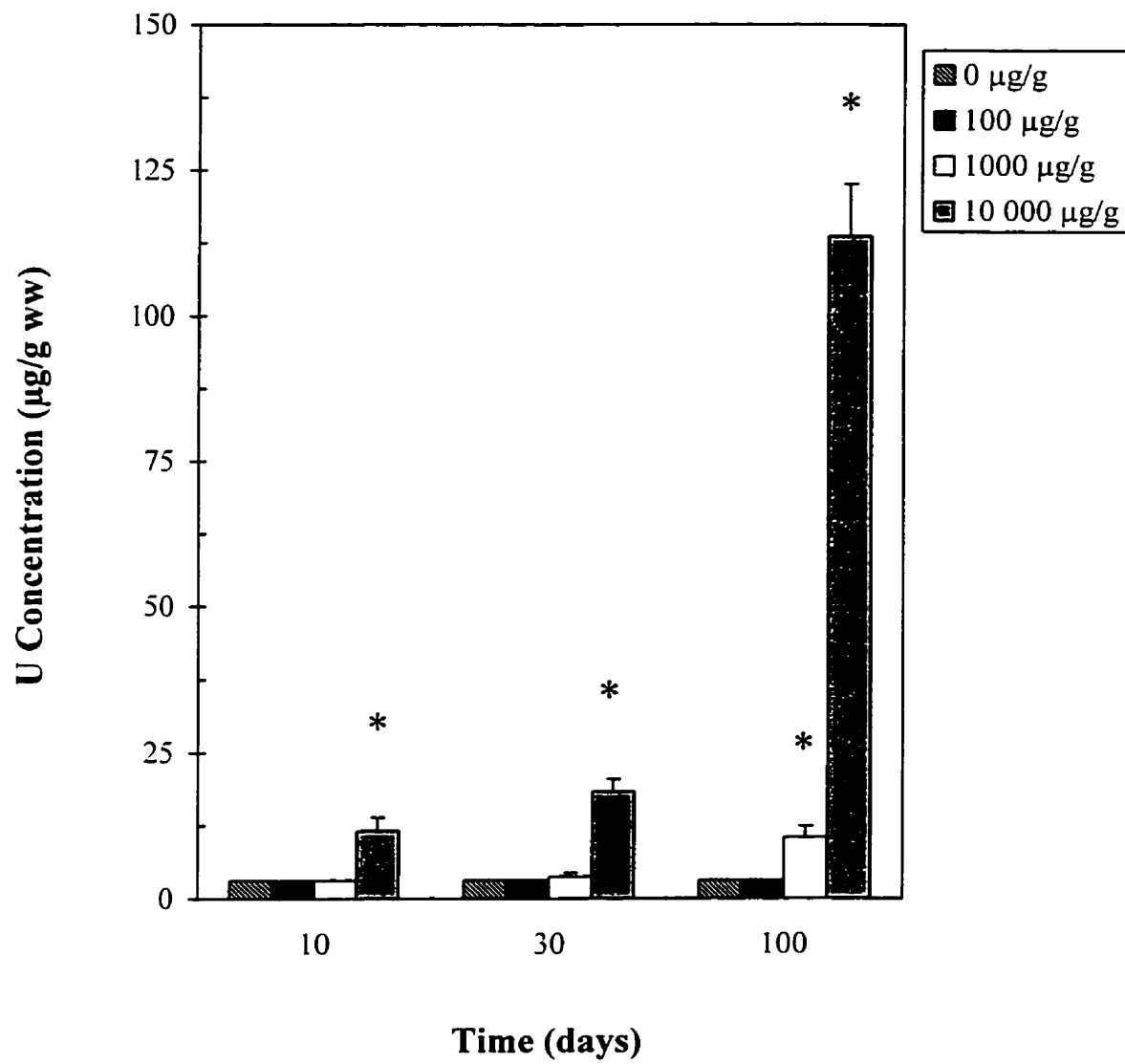
(B) Gill
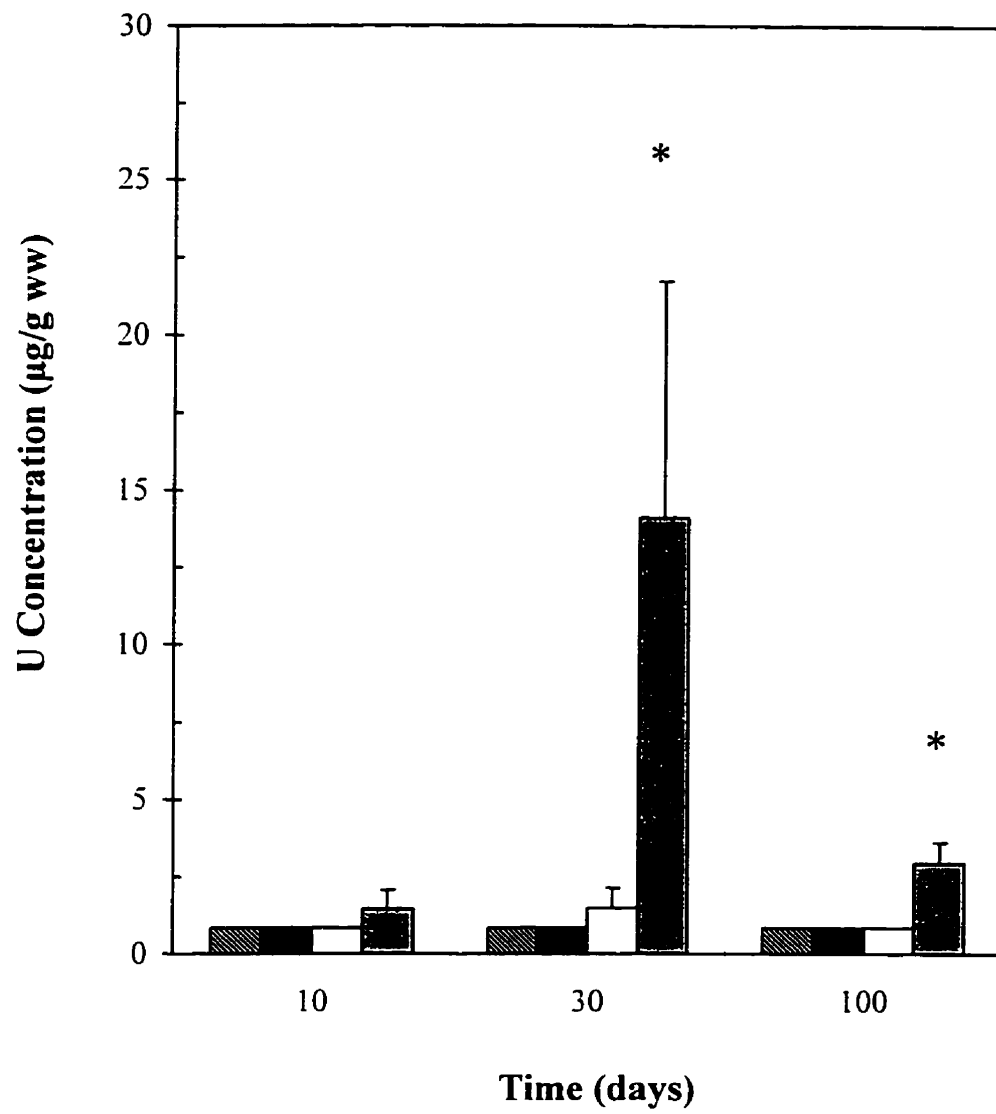
(C) Gonad
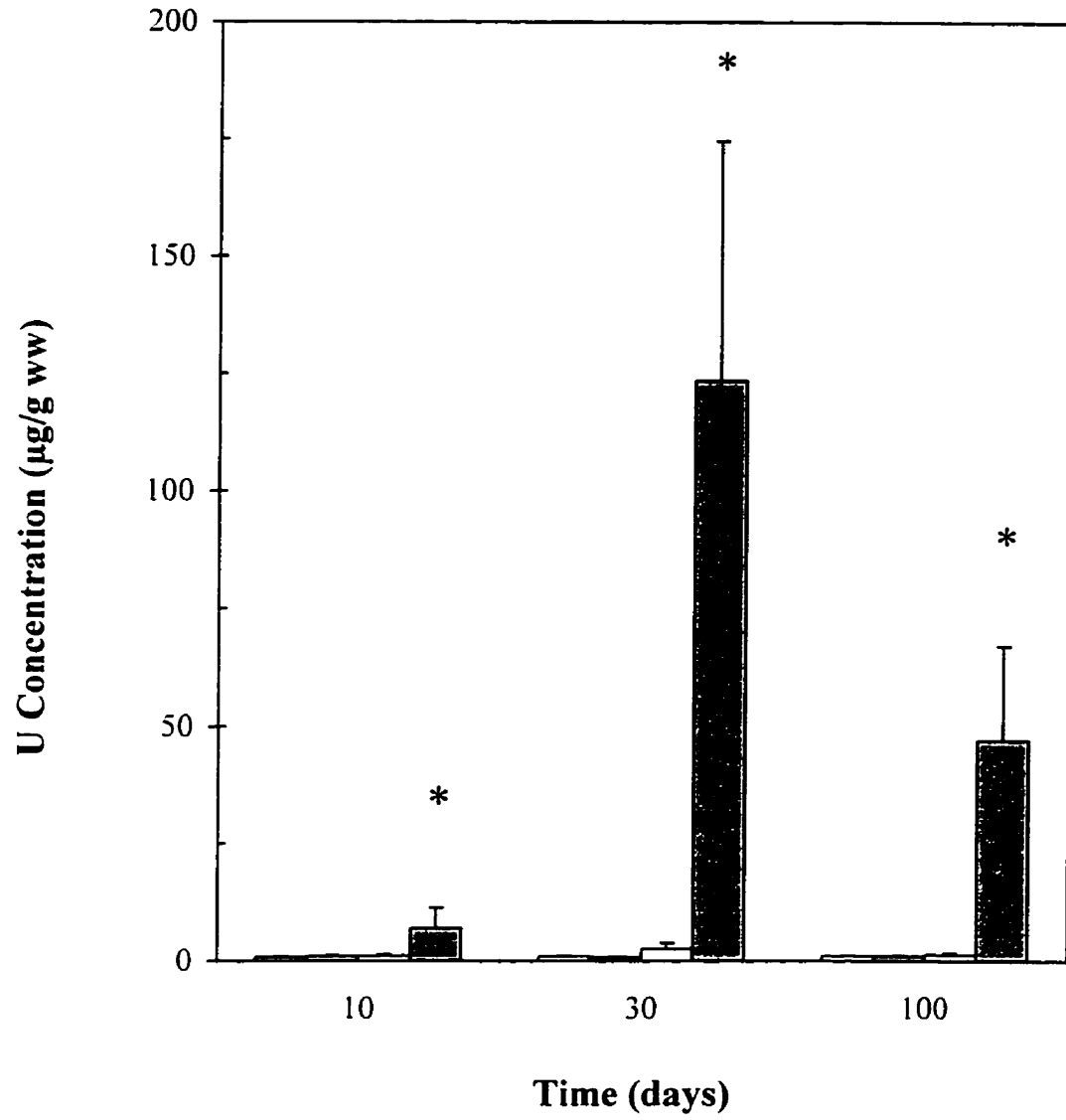
(D) Intestine
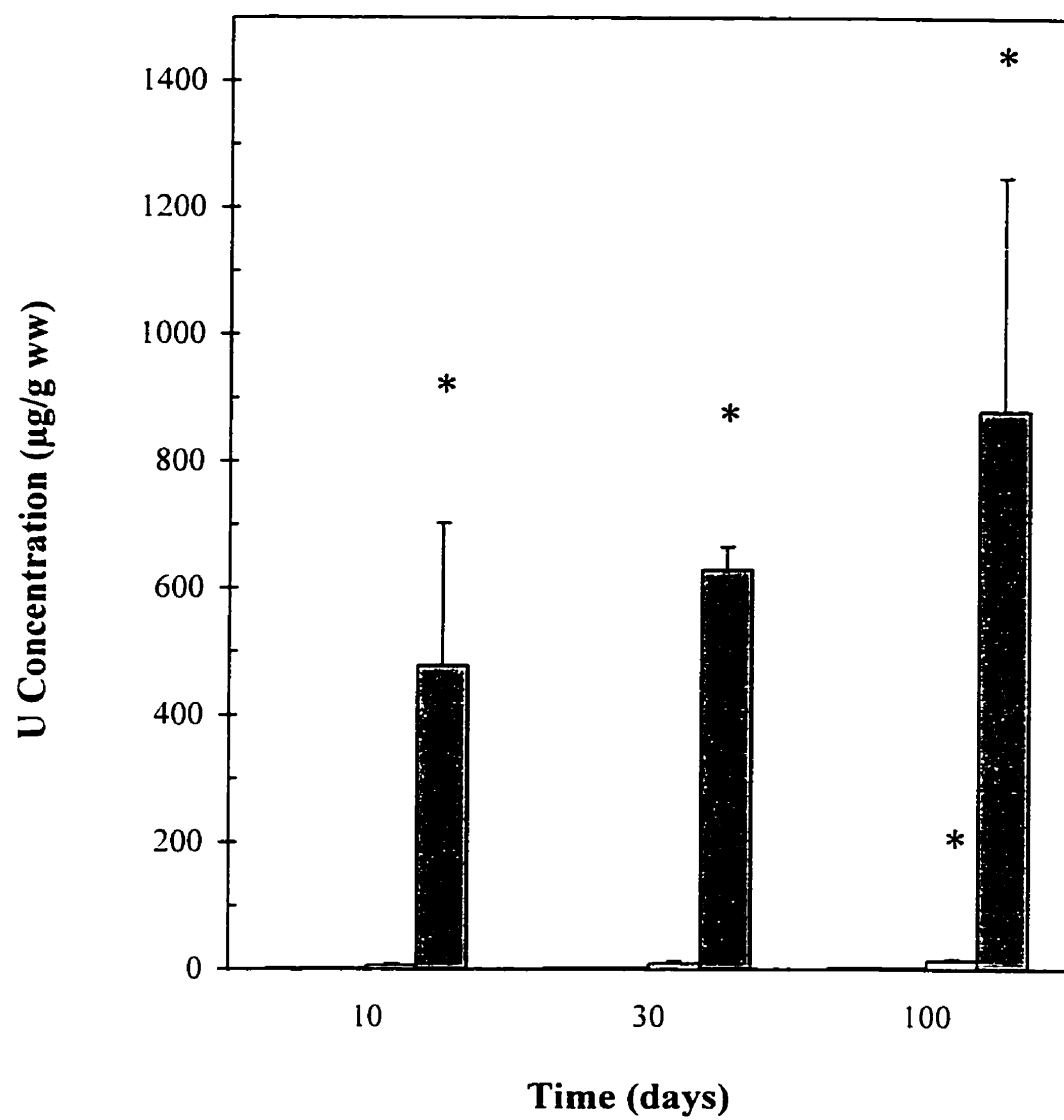
(E) Kidney
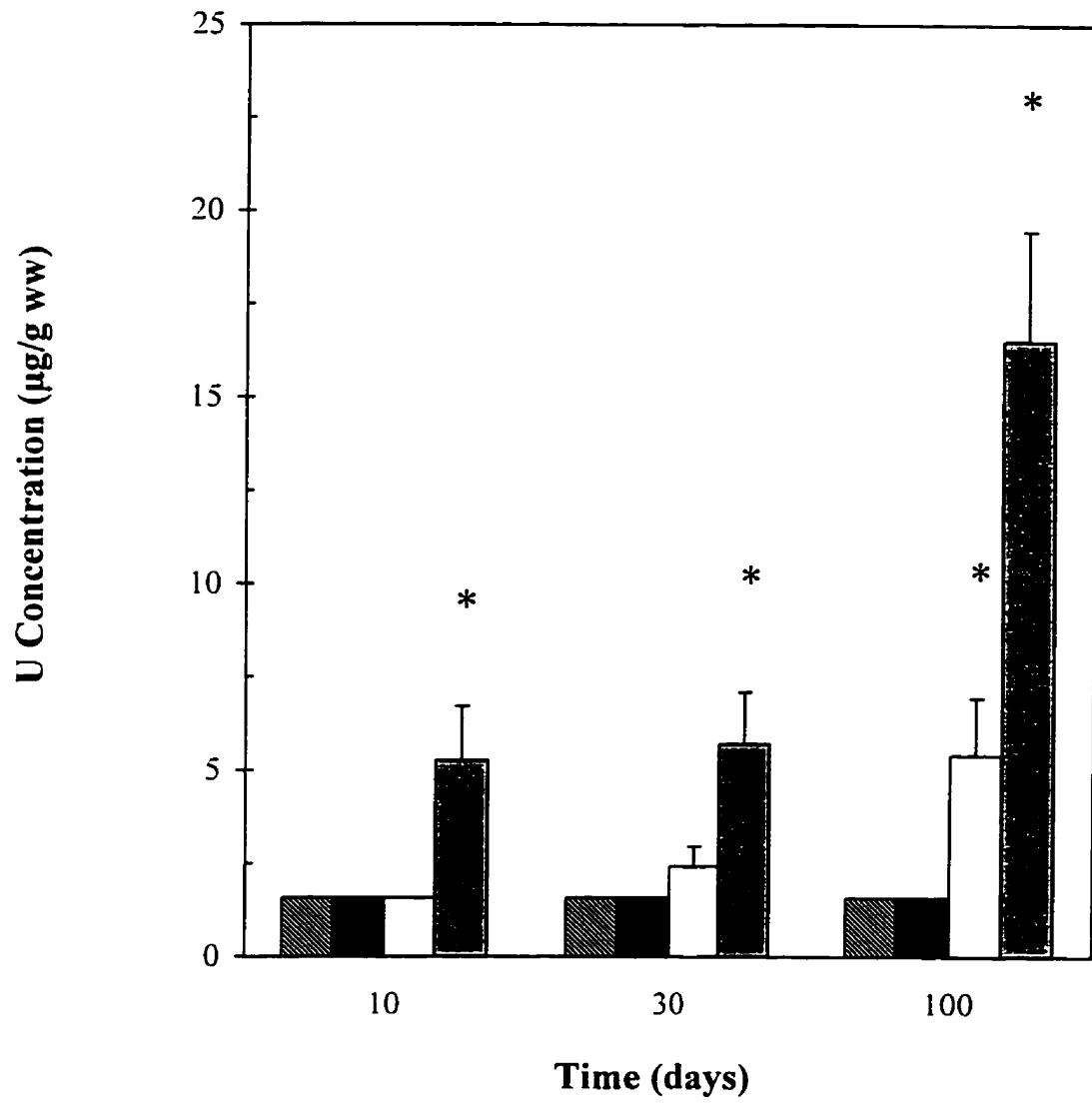
(F) Liver
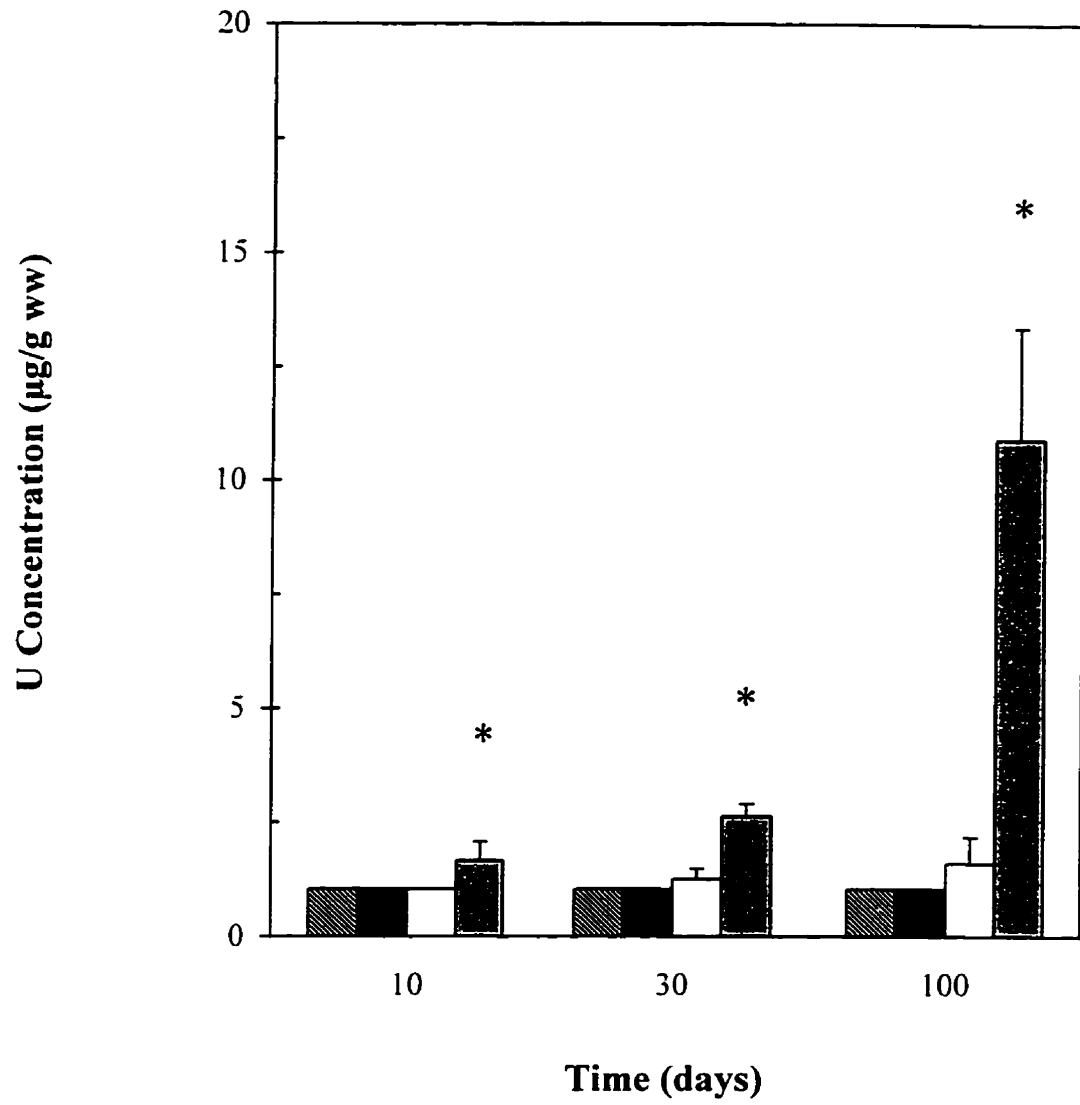
(G) Muscle
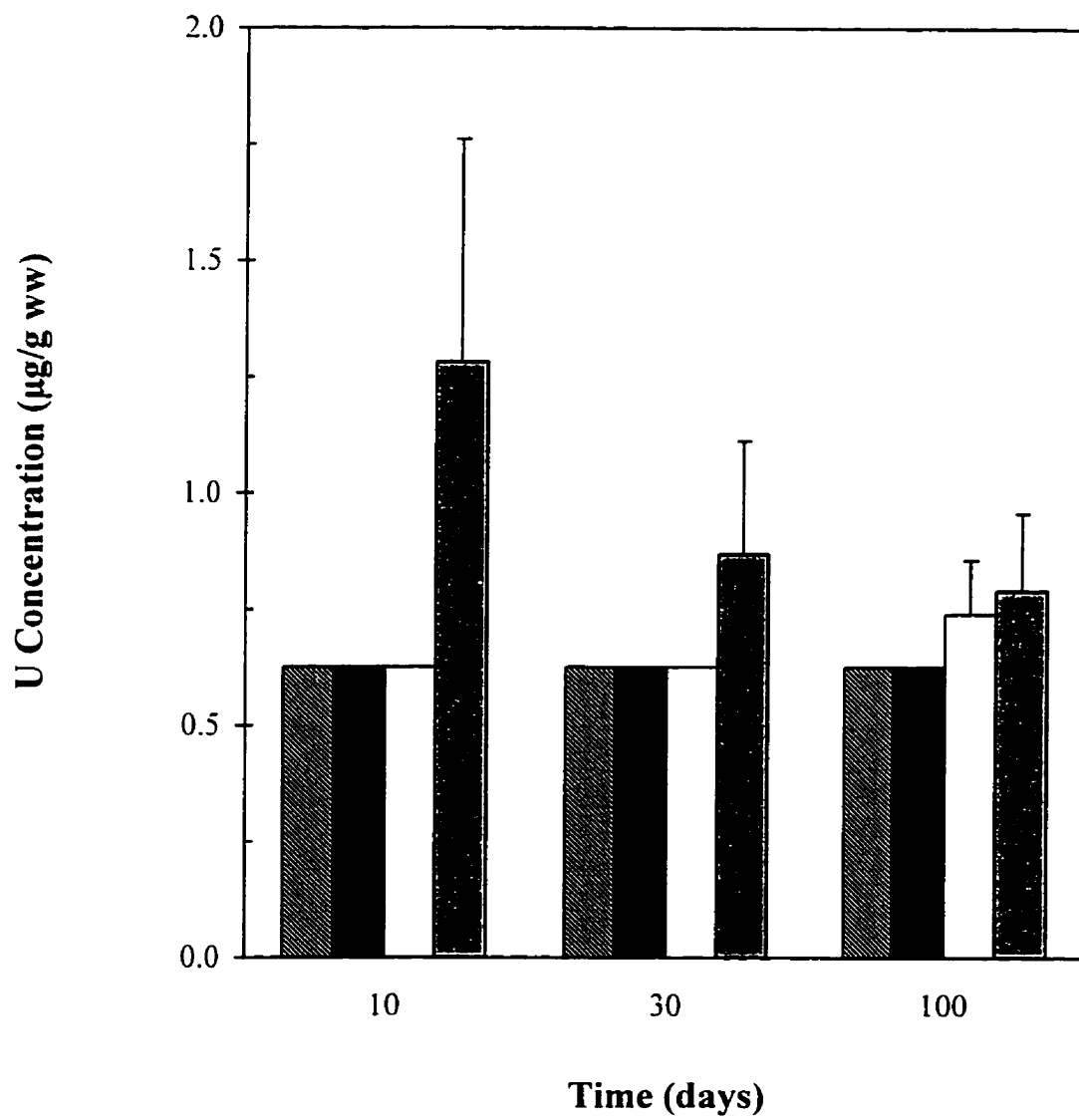
(H) Scales
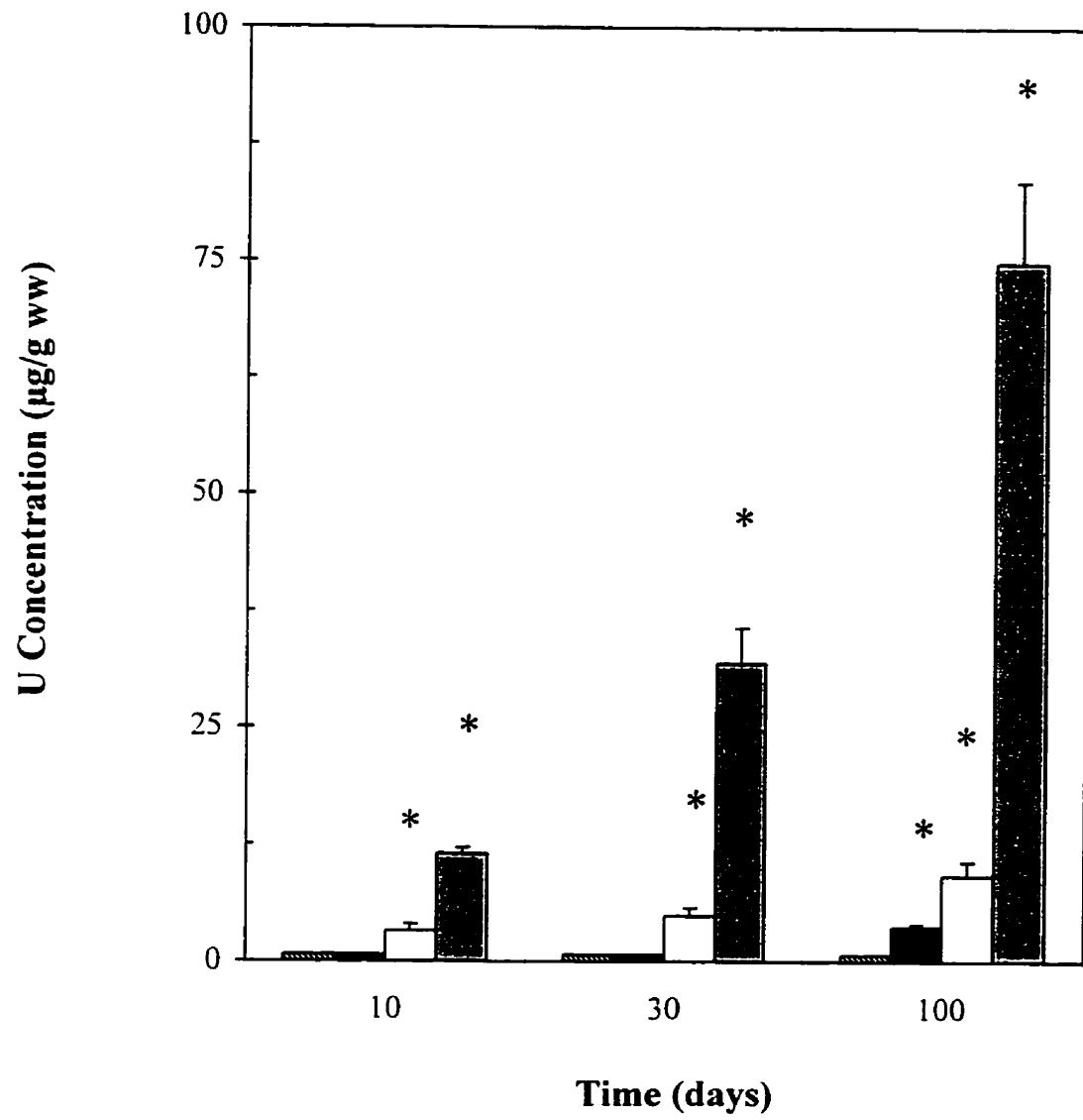
(I) Skin
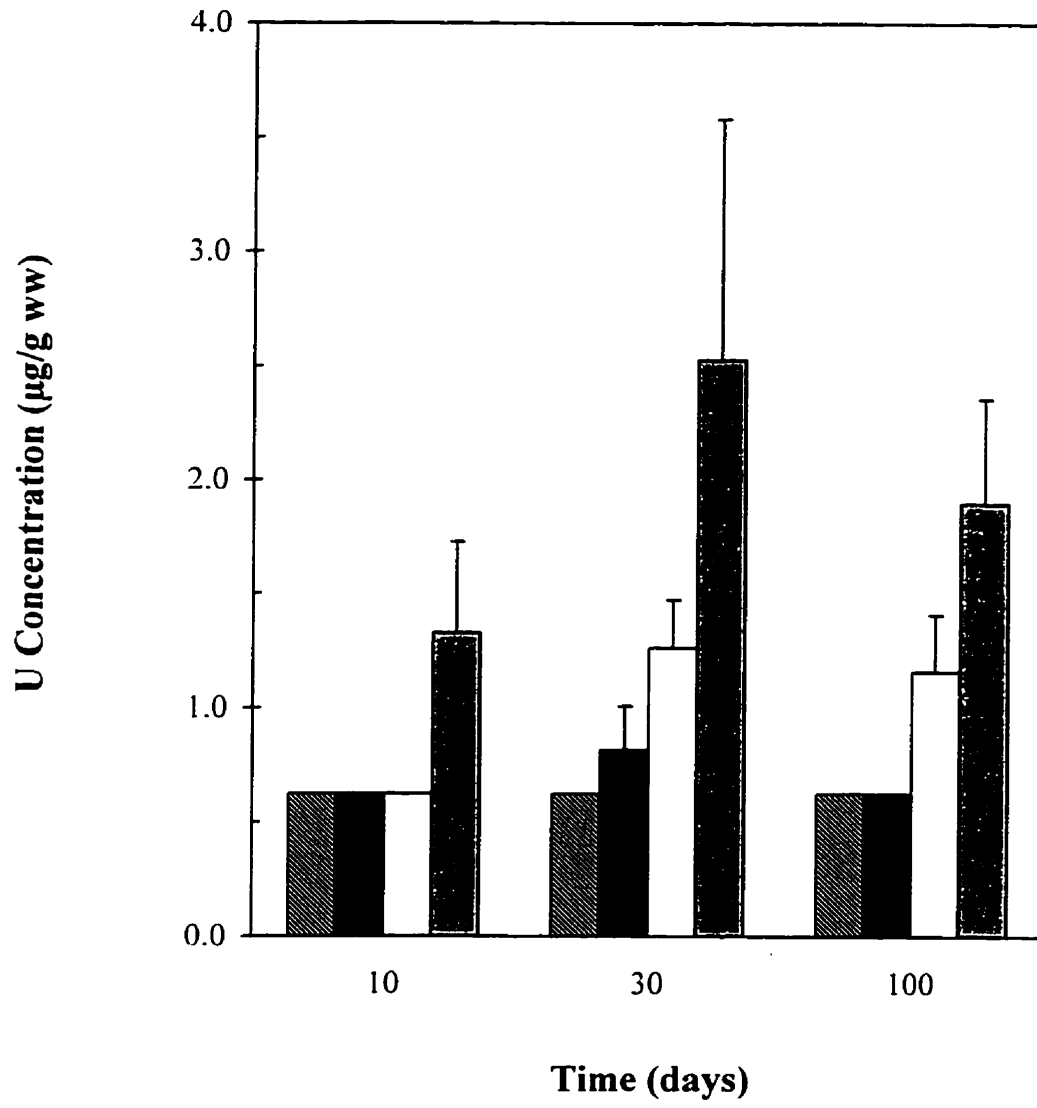
Figure 2.3. The distribution of U in nine tissues of lake whitefish fed 10 000 µg U/g food

for 10, 30, and 100 days. Data are expressed as mean (± SE).
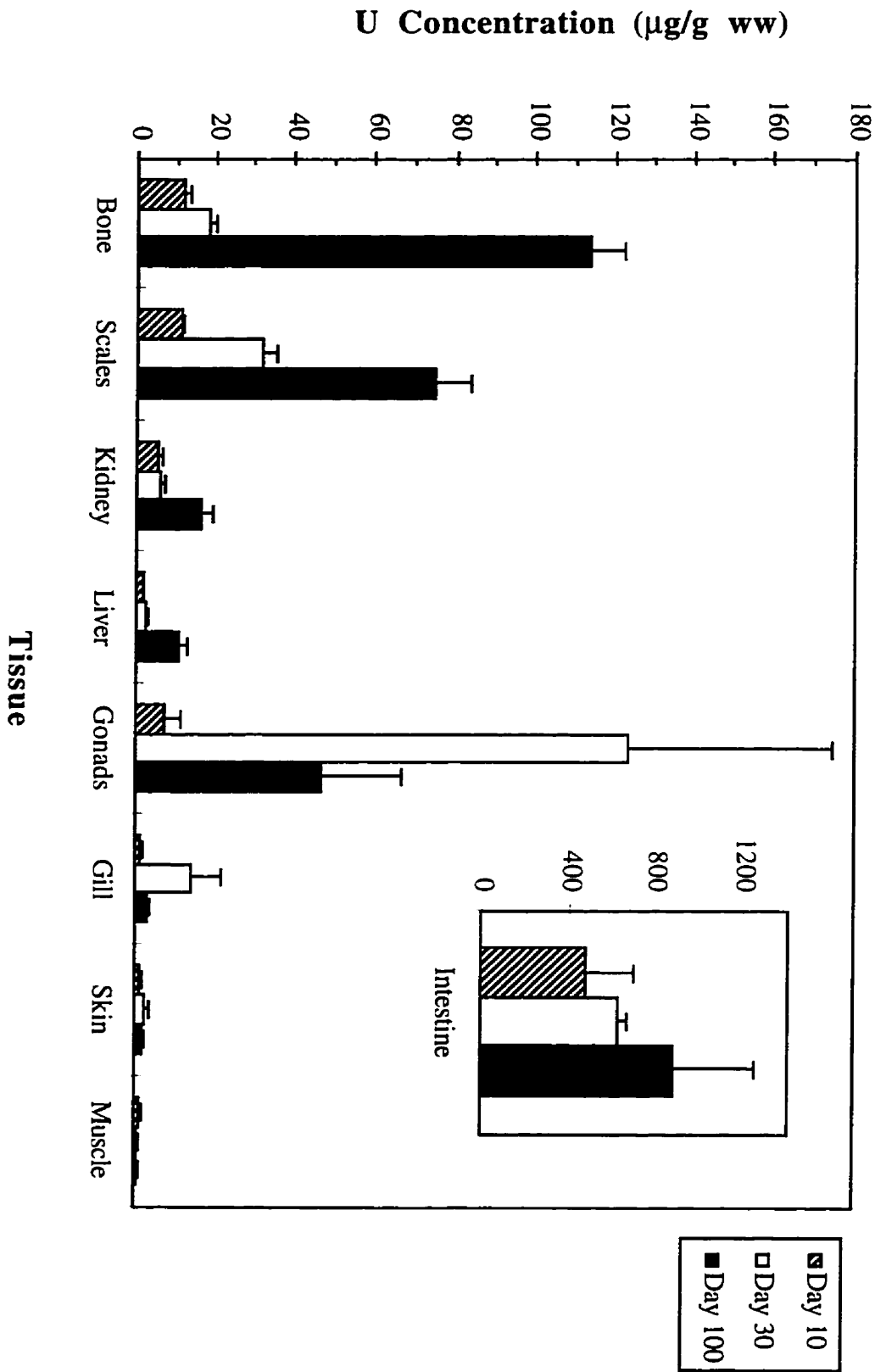
Figure 2.4. The accumulation of U, expressed as mean (± SE), in nine tissues of lake whitefish as a function of the total mass of U consumed: 284 µg U/g fish in fish consuming 1000 µg U/g for 100 days vs. 307 µg U/g fish in fish consuming 10 000 µg U/g for 10 days. Data from lake whitefish fed an uncontaminated diet are included for reference.
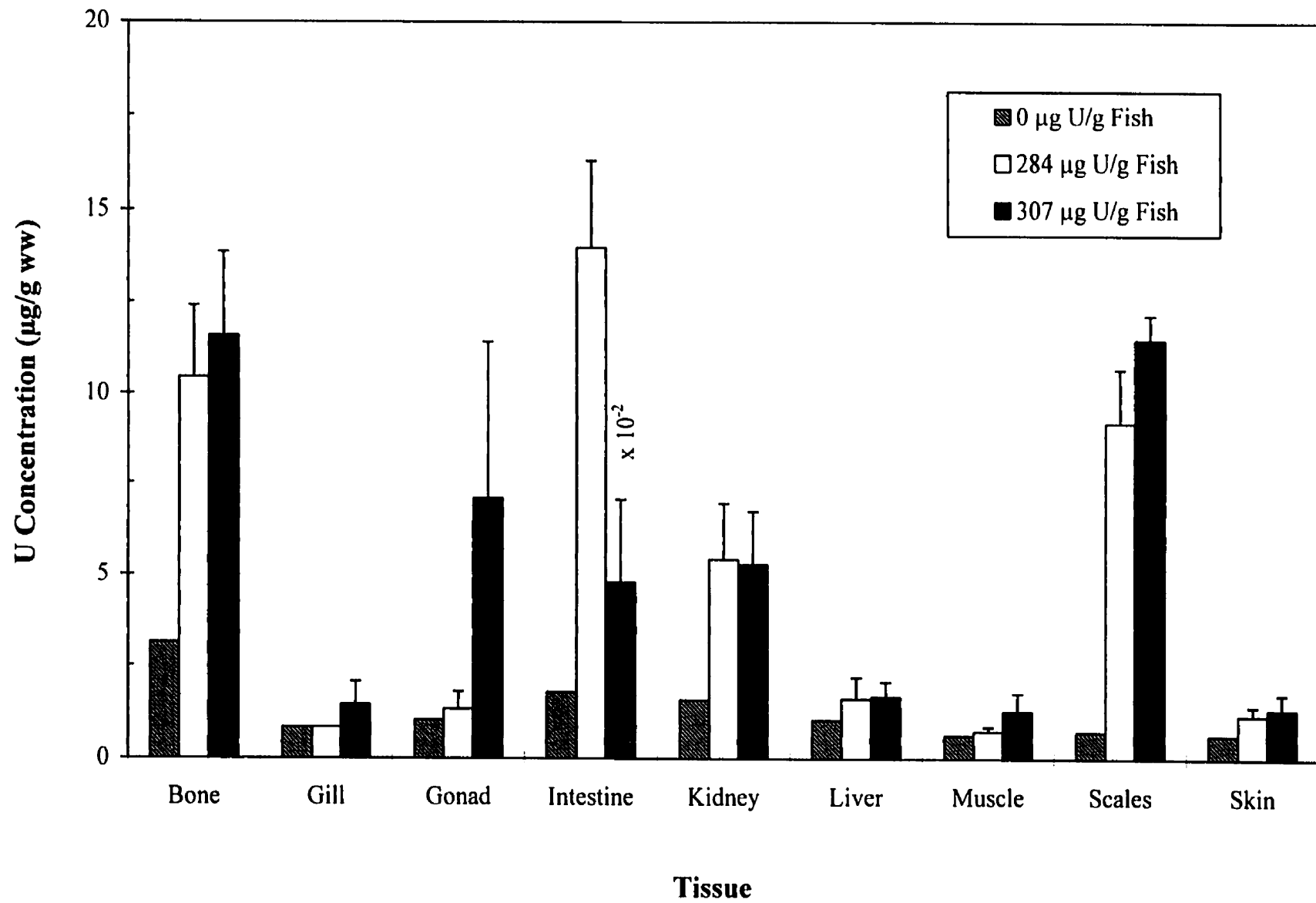
Figure 2.5. Linear regressions of the accumulation of U in (A) bone, (B) kidney, (C) scales, and (D) liver of lake whitefish fed 10 000 µg U/g as a function of time. Liver and scale data are log transformed. $P < 0.05$.

(A) Bone
$R^2 = 0.9146$

(B) Kidney
$R^2 = 0.5552$

(C) Scales
$R^2 = 0.9258$

(D) Liver
$R^2 = 0.7586$
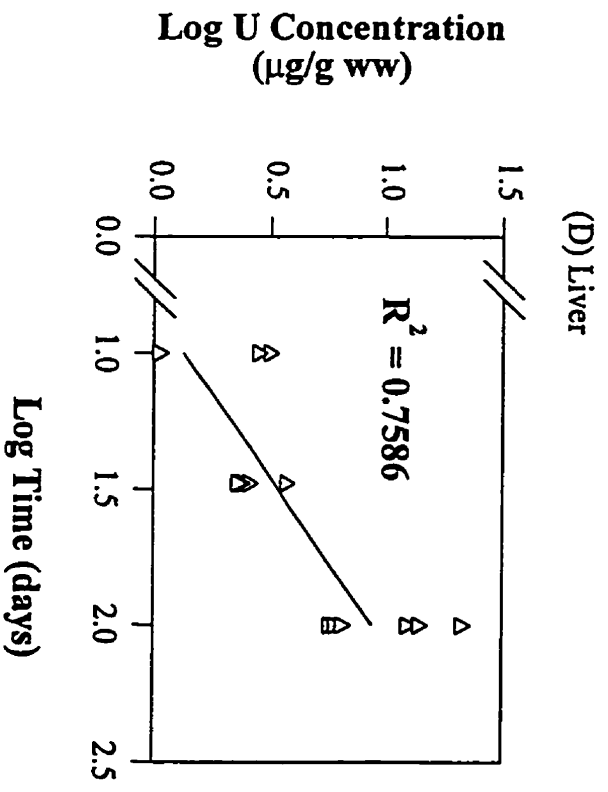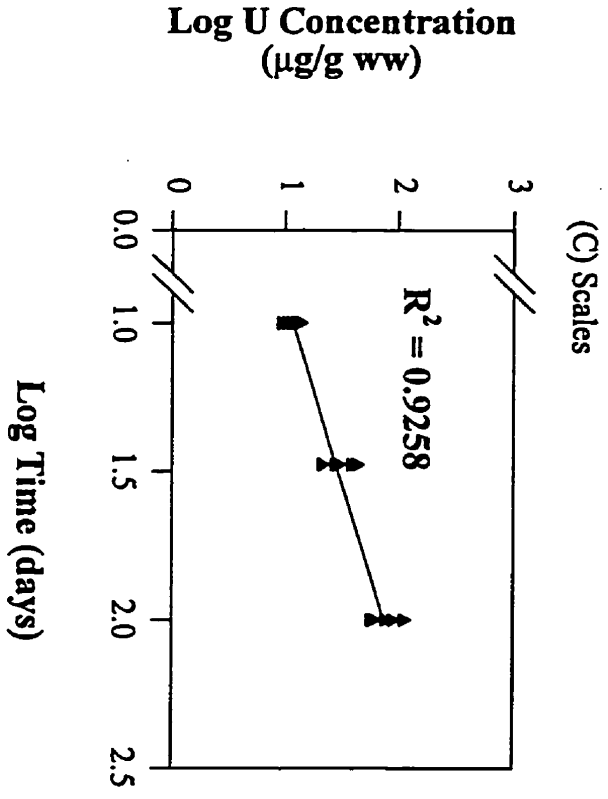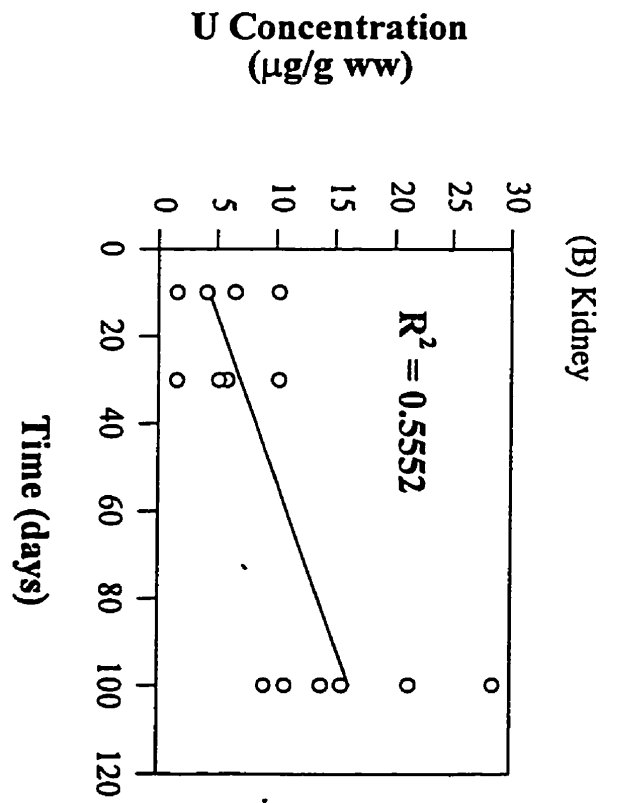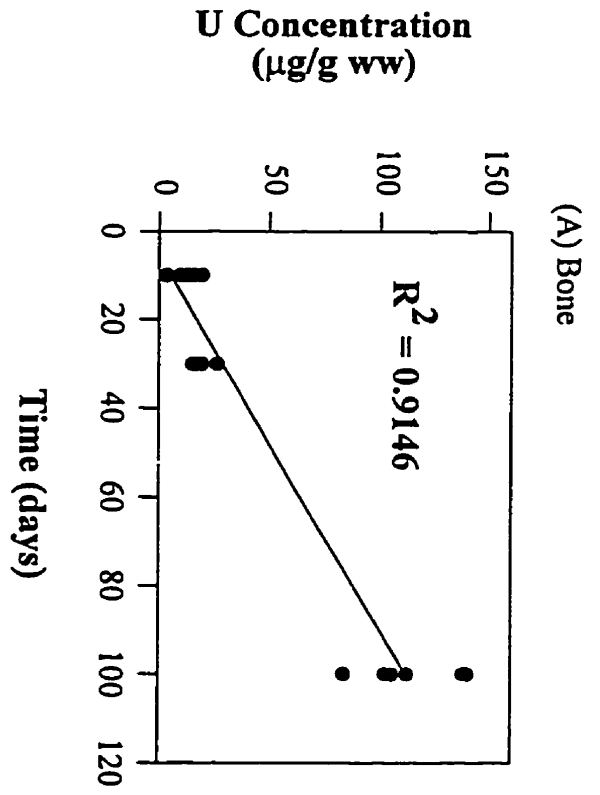
Figure 2.6. Linear regression of the accumulation of U in scales of lake whitefish fed

1000 μg U/g as a function of time. P < 0.05.
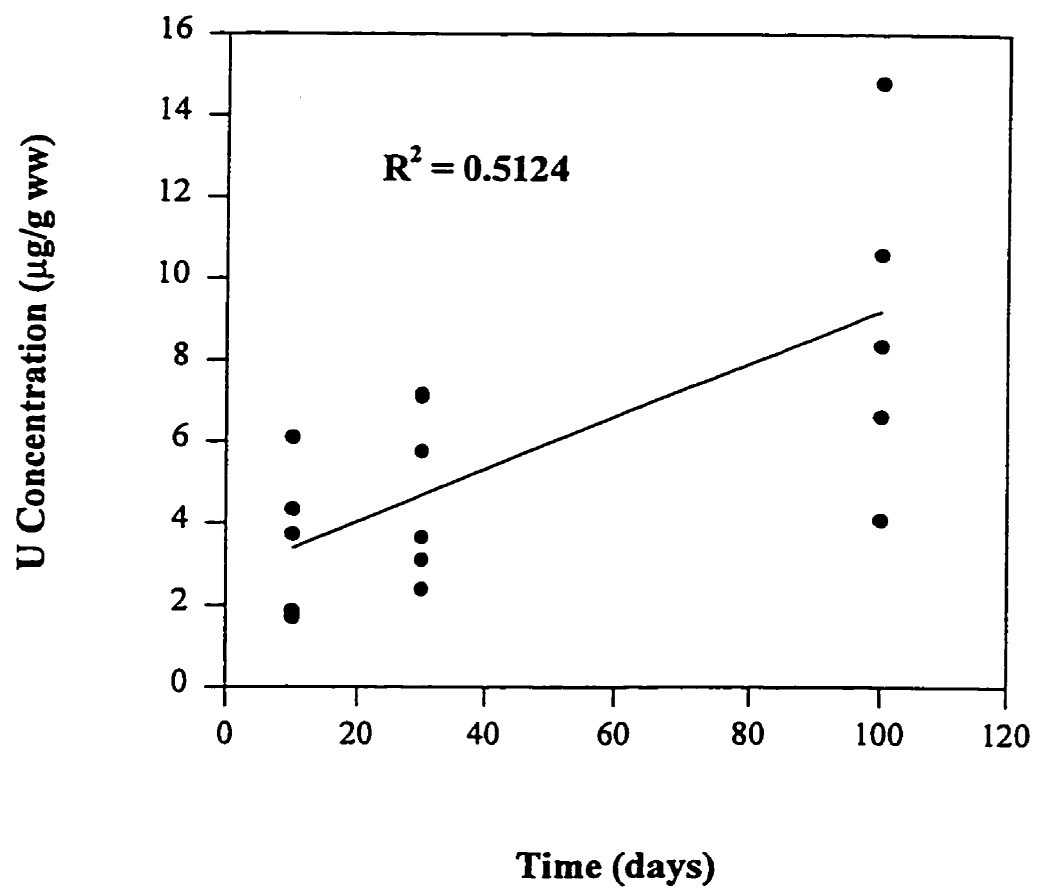
Figure 2.7. Linear regression of the accumulation of U in scales of lake whitefish fed U-contaminated diets sampled on day 100, as a function of exposure concentration: low, moderate, and high treatment groups. Data are log transformed. $P < 0.05$.
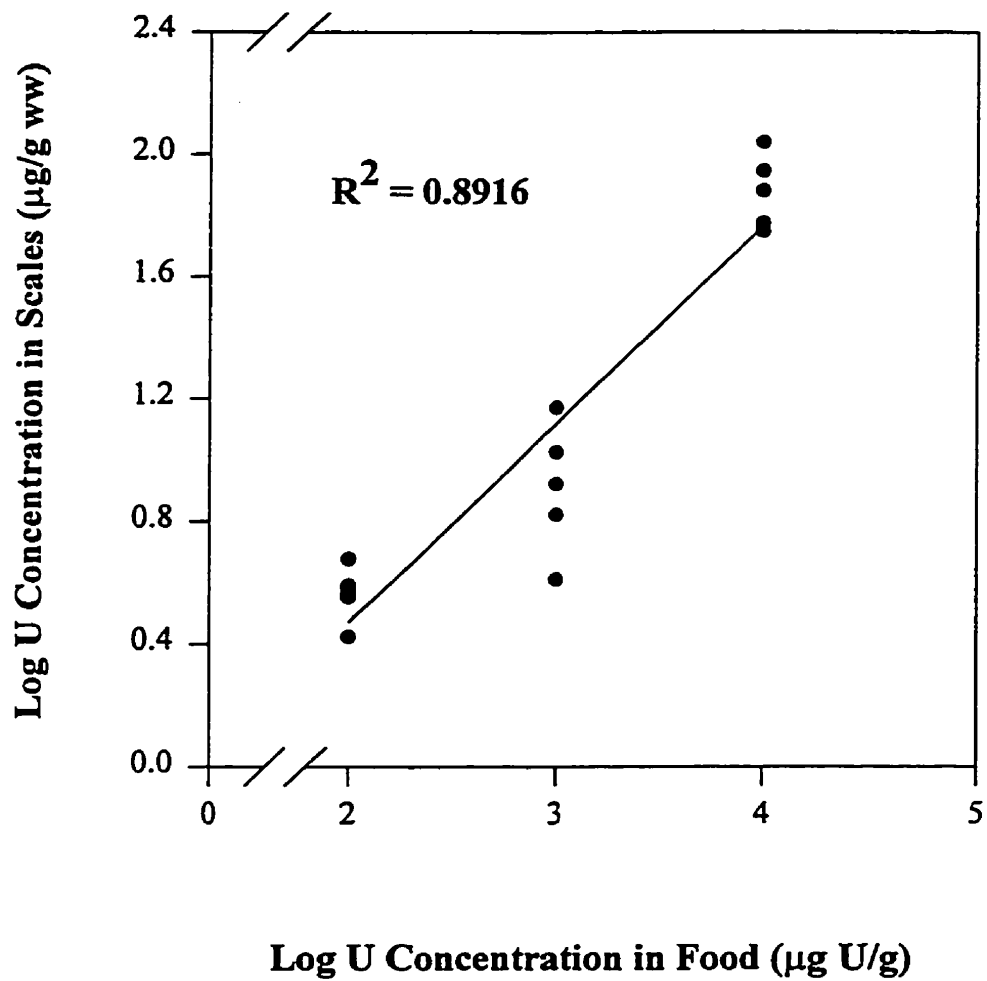
Figure 2.8. Linear regression of the accumulation of U in the scales of lake whitefish, fed diets contaminated with U, as a function of total mass of U ingested. Log transformed data were analyzed using the concentrations of U in the scales of individual fish fed diets contaminated with U. All data from the low, moderate, and high treatment groups were included, with the exception of fish fed 100 µg U/g for 10 and 30 days, in which no significant accumulation of U in scales was observed. $P < 0.05$.
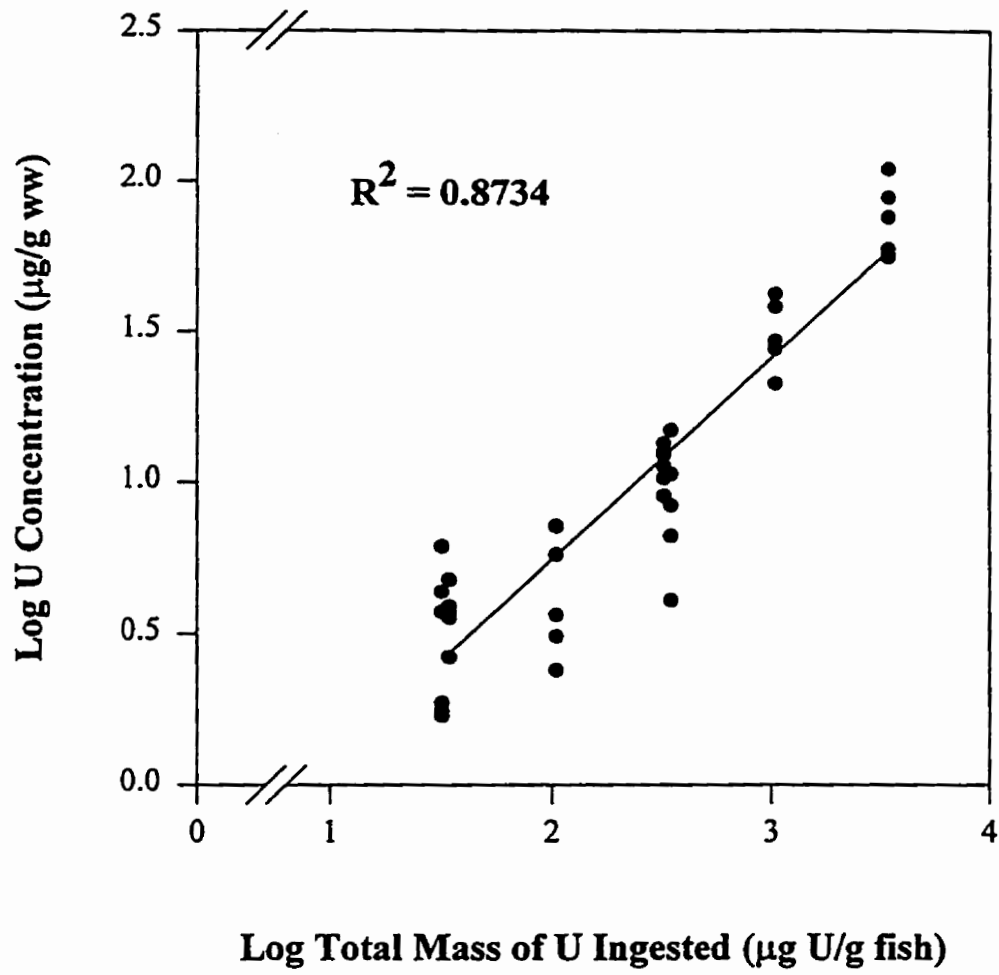
$R^2 = 0.8734$

Chapter Three

The adverse effects of dietary uranium in lake whitefish (*Coregonus clupeaformis*).

Abstract

Because U mining and milling activities cause U enrichment of aquatic systems, it is relevant to determine the potential for U to produce adverse effects in fish. To address this issue, a suite of indices of toxicity were evaluated in adult lake whitefish fed a commercial diet contaminated with three concentrations of U, 100 µg U/g, 1000 µg U/g, and 10 000 µg U/g, for 10, 30, and 100 days. Whole organism morphometrics were unaltered by U exposure. Haematological variables were either unchanged or only transiently affected. Small changes were observed in liver and kidney metallothionein concentrations. Concentrations of serum lipid peroxides were significantly elevated in all treatment groups on days 30 and 100, indicating that U may damage cellular and sub-cellular membranes. Dose- and duration-dependent histopathologies were present in liver and posterior kidney of treated lake whitefish. The most consistent and pronounced lesions in liver were focal hepatocyte necrosis and alterations of bile ductule epithelium. Dose- and duration-dependent renal lesions were most evident in proximal tubules. However, numerous other pathologies were observed in kidney including: tubular necrosis, inflammation, haemorrhaging, depletion of haematopoietic tissues, alterations of distal tubules and collecting ducts, tubule dilation, pigmented macrophage proliferation, and glomerular lesions. All concentrations of dietary U resulted in significant pathologies in lake whitefish following prolonged exposure. The latter indices of toxicity would be useful for assessing fish health in U biomonitoring programs.

I. Introduction

As discussed in detail in chapter one, significant quantities of U are discharged directly into watersheds neighbouring U mining and milling operations. Once introduced into aquatic ecosystems, uranium accumulates in the sediments (Hynes et al. 1987, Joshi et al. 1989, McKee et al. 1987, Neame et al. 1982, Swanson 1985, Waite et al. 1988, 1989) where it may be bioavailable to fish.

As demonstrated in chapter two of this study, U is absorbed in the gastrointestinal tracts of lake whitefish (*Coregonus clupeaformis*) fed contaminated diets and accumulates primarily in bone, scales, intestine, kidney, liver, and gonads. Although in most natural systems, the diet is recognized as the most relevant route of metal uptake in feral fish exposed over chronic periods (Campbell 1994, Handy 1996, Harrison et al. 1990, Kashulin and Reshetnikov 1995, Miller et al. 1992), there remains a paucity of data regarding the effects of metals administered orally to fish (Handy 1996).

Information regarding the sub-lethal toxicity of U in fish, particularly following chronic low-level exposure, is also scarce. Current knowledge of the hazards of U to fish is limited almost exclusively to acute lethality data generated from aqueous exposures (Buhl and Hamilton 1996, Bywater et al. 1991, Hamilton 1995, Hamilton and Buhl 1997, Holdway 1992, Parkhurst et al. 1984, Poston et al. 1984, Vinot and Larpent 1984). In contrast to research on the effects of most of the metals, metalloids, and radionuclides associated with U mining and milling discharges, laboratory assessments of the sub-lethal effects of U in fish are virtually absent.

In light of the current mining boom in Canada, it is important to evaluate the deleterious effects of U on fish health using an environmentally relevant exposure regime in order to assess potential risk to feral fish residing in impacted systems. Studies of the toxicity of waterborne U are of limited environmental relevance and contribute little to our understanding of the biotic hazards associated with this contaminant because the diet is the primary route of U exposure to feral fish (Emery et al. 1981, Kovalsky et al. 1967, Swanson 1982, 1983, 1985).

The second segment of this study was designed to evaluate some of the potential adverse effects of dietary U in lake whitefish. A suite of biomarkers of U exposure and toxicity spanning several levels of biological organization were evaluated, as has been recommended for assessments of fish health (Adams et al. 1990, 1996, Depledge et al. 1995, Holdway et al. 1995, Livingstone 1993). The general morphometric parameters examined at the whole organism level were: weight, length, condition factor (CF), and growth. At the organ and tissue levels, liver somatic index (LSI), haematological parameters, and histopathology of liver and posterior kidney tissue were evaluated. Finally, concentrations of metallothionein (MT) in liver and kidney, and lipid peroxides (LPO) in serum were measured. The indices of toxicity that were incorporated into this study were selected based upon the literature pertaining to mechanisms of effects of U in mammals discussed in chapter one.

II. Materials and Methods

Details on fish holding, tank parameters, dosing, food preparation, and fish sampling are provided in the Materials and Methods section of chapter two. Information on materials and methods relevant to this chapter is given below.

Haematological Sampling

A sample of blood was drawn from the caudal vein, after anesthetization of fish in pH-neutralized tricaine methane sulfonate (390 mg/L), using a 21 1/2 gauge needle and a 10 cc syringe pre-rinsed with ammonium heparin. A portion of blood was transferred to haematocrit capillary tubes, sealed, and centrifuged at 8000 rpm for 5 min using a Damon/IEC Division Clinical Centrifuge (Needham, MA). Haematocrit was measured using a Damon/IEC Division micro-capillary haematocrit reader (Needham, MA). The remainder of whole blood was transferred to non-heparinized vacutainers and centrifuged for serum separation. Serum was pipetted in several sub-samples into 1.5-mL microcentrifuge tubes and frozen at -90°C for later haematological analysis. One of each of these replicate subsamples was held at this temperature until analyzed for LPO.

Histological Sampling

Liver was weighed after the gallbladder was carefully dissected free. A piece (approximately 5 mm^3) of liver and posterior kidney from the mid region were excised for histopathological analysis. Both tissues were placed in freshly prepared Bouin's fixative, at a volume of no less than 20 mL for every fish, within 5 min of sacrificing.

Tissues were fixed for 48 h, rinsed in five changes of 70% ethanol over a 24-h period and stored in 70% ethanol until further processing.

Biochemical Sampling

Following removal of histological samples, the liver was frozen for biochemical analysis. The kidney was excised after removal of the swim bladder and overlying mesentery. Lateral incisions were made with a scalpel along the body wall on either edge of the kidney and it was removed with a spatula. Liver and kidney were stored frozen at -90°C for biochemical analyses.

Haematological Analyses

Serum was stored at -90°C until analyzed for concentrations of Cl^-, K^+, and Na^+, and serum osmolality. Serum chloride was analyzed with a Corning model 925 chloride analyzer. Serum potassium and sodium were analyzed using an Instrumentation Laboratory model 943 flame photometer. The osmolality of duplicate 50-μL samples of serum were analyzed using a Precision Systems model 5004 μOsmette®. Haematocrit was analyzed immediately following blood withdrawal using a Damon/IEC Division Micro-capillary haematocrit reader (Needham, MA), as described earlier.

Metallothionein Analysis

Liver and kidney were analyzed for metallothionein using a modified method (J.F. Klaverkamp, The Freshwater Institute, 501 University Crescent, Winnipeg, Manitoba,

Canada, R3T 2N6; personal communication) of the mercury saturation assay of Dutton et al. (1993). Briefly, a cold solution of 0.9% NaCl was added to duplicate samples of 0.3 to 0.5 g of kidney and 0.2 to 0.4 g of liver in a ratio of tissue: saline equal to 1:3 and 1:5, respectively. The samples were homogenized, using a Brinkman polytron homogenizer with a 90/Polytron PTA 10-S generator, on ice. One gram of the homogenate was heat treated for 5 min at 95°C and cooled in an ice bath for 5 min. Samples were centrifuged at 10 000 rpm for 5 min (Eppendorf model 5412, Mandel, Edmonton, Alb.) and the supernatant was removed and frozen at -90°C for metallothionein analysis.

For the assay, 200 µL of a ^{203}Hg working solution was added to 200-µL of sample supernatant, the sample was mixed by vortex, and incubated at room temperature for 10 min. The working solution consisted of 5 µL/mL of cold $HgCl_2$ stock solution at a concentration 10 mg Hg/mL of 20% tricholoroacetic acid (TCA), and a sufficient volume of ^{203}Hg (Amersham) to arrive at a final specific activity of 500 000 cpm/10 mls of stock, once diluted with a 20% TCA solution. Following incubation, 400 µL of a solution of one part egg albumen homogenized in one part 0.9% saline was added, the samples were mixed by vortex, and centrifuged at 10 000 rpm for 3 min. A 500-µL aliquot of the supernatant was transferred to 600-µL microcentrifuge tubes for gamma counting. Gamma radiation from the ^{203}Hg was analyzed using a LKB-Wallac 1282 Compugamma gamma counter (Fisher, Winnipeg, Man.).

Metallothionein standards (rabbit liver MT II, Sigma Chemical Co., St. Louis, MO), analytical blanks, and total counts (i.e. working stock) were run concurrently with samples to ensure analytical accuracy and for the construction of standard curves. In

addition, an internal reference sample of lake whitefish liver, prepared at the Freshwater Institute, was run in duplicate. Briefly, a liver from a reference lake whitefish was prepared as above, and ten, 200-μL replicates of the supernatant were analyzed for MT, using the same procedure described above, to establish a baseline. Concentrations of MT were accepted when the readings for the internal reference sample fell within the established range of variation.

Analysis of Serum Lipid Peroxides

Serum LPO were analyzed using the Serum LPO-CC kit (Kamiya Biomedical Company, Thousand Oaks, CA). All reagents used in the procedure were provided in the LPO-CC kit. Serum samples were kept frozen at -90°C until analysis. For each sampling day, the assay was performed precisely 14 d later to control for the age of the samples as a factor in the spontaneous production of lipid peroxides in serum. Samples were removed from the freezer and thawed on ice for 15 min in the dark. An aliquot of 50 μL of serum in duplicate was then immediately run through the assay.

Histological Processing

Tissues were processed in an automated tissue processor (IL MVP Tissue Processor) using an ethanol/butanol series. Liver and kidney samples were embedded in paraffin (Tissue Prep II) and sectioned at 6 μm and 7 μm, respectively. Tissue sections were mounted on glass slides and stained with haematoxylin and eosin for histopathological analysis. The procedure, described in Edwards (1950), involved

paraffin removal, rehydration, staining in Harris' haematoxylin, differentiation in acid alcohol, treatment in solutions of 0.33% phosphotungstic acid and 0.33% sodium citrate, counterstaining in aqueous Eosin Y, dehydration, and clearing. Number 1 1/2 glass coverslips were mounted on slides with Cytoseal 60 mounting medium (Stephens Scientific, Riverdale, NJ). All chemicals were obtained from the Fisher Scientific Company (Fair Lawn, NJ), with the exception of phosphotungstic acid which was obtained from the Sigma Chemical Co. (St. Louis, MO).

Photomicrographs were taken using a Zeiss Photomicroscope III with a built in camera, using Ilford PAN F Plus 135 film (ISO 50/18° DX). Film was developed and printed on Ilford 5.1P Ilfobrom 5 glossy, single weight photographic paper and Kodak Polycontrast III RC Paper. Photographs of labelled and mounted photomicrographs were taken using Kodak TMAX 100 (ISO 100/21° DX) film and printed on 8 1/2 by 11 in sheets of Kodak Polycontrast III RC Paper.

Analysis of Histopathology

Liver

Liver was assessed by three approaches: qualitative, semi-quantitative, and quantitative analysis. In all assessments, the centre-most tissue section of liver on a slide made from each fish was selected for evaluation. For qualitative analyses, a section of liver was thoroughly examined for the presence of lesions.

A semi-quantitative approach was employed for estimations of the extent of hepatocyte necrosis and tissue structural changes. The damaged proportion of a section of liver from each fish was estimated subjectively and ranked accordingly. Four categories were used to describe the affected fraction, expressed as a percentage: (1) negligible (< 1 %), (2) minor (1 - 5%), (3) moderate - severe (5 - 30%), and (4) massive (> 30%). Tissue alterations included: pyknotic and ruptured (i.e. karyorhectic) nuclei, cell separation, the presence of cell debris, cell lysis and sloughing, altered nuclear morphologies, cytoplasmic deposits, lipid infiltration, and markedly enlarged hepatocytes. The first category was deemed 'normal' because it accounted for livers with a negligible degree of hepatocyte turnover, a non-pathological observation in this tissue.

Hepatocyte morphometrics were obtained by projecting microscopic images onto a digitizer (Summagraphics Bit Pad, Fairfield, CT) using the SigmaScan (version 3.90) software package (Jandel Scientific, Corte Madera, CA). For each fish, the diameters of hepatocyte nuclei were measured in the first 50 cells with spherical nuclei that were encountered while moving downward from the top of the section. Relative hepatocyte size (i.e. area) was estimated by counting the number of hepatocyte nuclei in two fields of standard area (18 000 μm^2) from the centre-most section on a slide. Nuclear areas were calculated, from the formula πr^2. Cytoplasm areas were obtained by subtracting nuclear areas from estimates of hepatocyte size. Nuclear area to cytoplasmic area ratios (N:C) were then generated from the latter values.

Posterior Kidney

Histopathology in posterior kidney was assessed by approaches similar to those employed for liver. The centre-most section of posterior kidney on a slide from each fish was thoroughly examined for alterations. Among the pathologies assessed was the abundance of pigmented macrophages (PM). A number of researchers have successfully measured the sizes and frequencies of pigmented macrophage aggregates (PMA) in various tissues of fish (Blazer et al. 1987, Couillard and Hodson 1996, Haaparanta et al. 1986, Wolke et al. 1985). However, because salmonids do not form discrete and organized aggregations of PM like most teleosts (Agius 1985), direct quantification of their abundance was not possible. Thus, the assignation of an increased abundance of PM in the posterior kidney of treated lake whitefish was made via thorough comparisons to abundances observed in control fish and was accepted only where there were clear increases in cell numbers.

Semi-quantitative evaluation was applied to the first (P1) and second (P2) segments of the proximal tubules. The first 15 cross sections of P2's (or P1's) encountered while moving downward from the top of the centre-most section on each slide, were categorized according to the degree of alteration observed. Four categories were used: (1) normal, (2) minor, (3) moderate, and (4) major. The parameters considered in the assignation were: staining, nuclear morphology and position, vacuolation and deposits in cytoplasm, brush border damage, lumen and tubule diameters, and debris in lumina. Criteria for each category were as follows: (1) no observed alterations, (2) some parameters slightly affected (e.g. alterations in one or two nuclei), (3) more cells were affected (i.e. > 2 but < half of epithelial cells) and/or the degree of alteration was more

marked (i.e. more structures affected) but was not severe enough to reasonably conclude that functioning was completely compromised and/or irreversible, and (4) tubules that were severely damaged (e.g. tubular necrosis) such that they were considered to be incapable of normal functioning. The data obtained for each fish were pooled within each treatment group and expressed as the frequency (from a total of 90 tubules) of tubules per treatment group assigned to each of the above categories.

Tubules which could not be confidently identified as P2's (e.g. tubules with characteristics intermediate between P2 and P1) were excluded and those displaying alterations intermediate between two ranking categories were assigned the less severe rank. Furthermore, where tubules were so severely altered as to be unidentifiable with reasonable certainty, they were excluded. Quantitative assessments in this tissue are described in Appendix one.

Statistical Analysis

All data were analyzed using the SigmaStat 2.0 Software Package (Jandel Scientific, Corte Madera, CA). Mean MT concentrations of each treatment group were compared to controls by the Student's t-test. Other data were analyzed by a One Way Analysis of Variance (ANOVA) within each block of time and within each dose. Specifically, this analysis was employed for analysis of treatment related effects on wet weight, fork length, CF, growth, LSI, and quantitative histological measurements. Where the conditions of normality and equal variance were not met, analysis was performed using the Kruskal-Wallis One Way ANOVA on Ranks. This latter method was employed

for evaluation of treatment effects on haematological variables and serum lipid peroxides. For both statistical methods, where differences were significant, treatment groups were compared to controls using the Dunnett's or Dunn's multiple comparison methods. Significance was tested for all statistical procedures using an $\alpha = 0.05$, unless otherwise indicated. Linear regression analyses were also performed on data for determining the strength of dose- and exposure duration-dependent effects where applicable (95% C.I.; $\alpha = 0.05$). CF was calculated using the formula: wet weight (g) / length^3 (cm^3) * 100. LSI was calculated using the formula: liver weight (g) / body weight (g) * 100. Data are presented as mean ± SE.

III. Results

Observations on feeding, behaviour, overt toxicity, and gross pathologies (i.e. necropsy) are provided in chapter one.

Morphometric Indices

The mean fish weights, fork lengths, and CF of each treatment group did not differ from controls on any sampling day (Table 3.1). Growth, expressed as a mean percentage increase in wet weight from the initial body weights, was similar amongst all groups on each sampling day (see chapter two). By the end of the exposure, there was a mean 17.2% increase in fish wet weights (all tanks combined) from the initial weighing.

With the exception of a reduction in the LSI of fish fed 10 000 μg U/g on day 100, the

LSI of treated fish did not significantly differ from controls (Figure 3.1).

Haematological Parameters

Little change in the haematological variables of haematocrit, serum osmolality,

Na^+ + Cl^- / osmolality, and serum Cl^-, Na^+, and K^+ occurred throughout the exposure

period (Table 3.2). The exceptions were elevations in the concentration of serum Cl^- in

the high and moderate treatment groups and in serum osmolality in the high treatment

group on day 30.

The nearly moribund fish (see chapter two) sampled on day 10 displayed

disturbances in all haematological variables examined. This individual had decreased

serum Na^+ (131.8 mM/L), Cl^- (93.5 mM/L), and osmolality (274 mOsm/L), increased

serum K^+ (3.68 mM/L), and a reduced haematocrit (29.5%).

Metallothionein

The concentrations of MT in whitefish liver and kidney are presented in Table

3.3. The mean concentration of MT in the liver of fish fed 10 000 μg U/g for 10 days was

significantly higher than controls. However, this induction did not persist. No other

differences in treated fish relative to controls were found in this tissue. With one

exception, a decrease in MT in kidney of lake whitefish fed 1000 μg U/g for 100 days, no

significant differences in the concentrations of MT in the kidney of fish fed contaminated

diets relative to controls, were observed.

Serum Lipid Peroxides

Concentrations of LPO in the serum of lake whitefish fed contaminated diets are presented in Figure 3.2. Significant elevations occurred in fish from all treatment groups on days 30 and 100. Strong dose-dependent increases in serum LPO were observed on day 30 ($R^2 = 0.5712$; $P < 0.00005$) and day 100 ($R^2 = 0.5516$; $P < 0.00005$).

Histopathology

Liver

Lake whitefish liver consists of polygonal hepatocytes typically arranged in cords (trabeculae) two cells-thick, separated by liver sinusoids (Figures 3.3A, C, and 3.4A). The sinusoids drain into central hepatic veins which in turn merge with the hepatic vein. Also present are small bile canniculi which converge with bile ductules and ultimately the bile ducts. Bile ductules and associated arterioles (Figure 3.5A) were observed in lake whitefish liver tissue sections.

Pathology observed in the livers of treated whitefish included: necrosis (Figure 3.3B,D), abnormal architecture (Figure 3.4B), clear foci of liver parenchyma (Figure 3.4D), and alterations of the bile ductule epithelium (Table 3.4, Figure 3.5B-D). The most severe lesions and the highest frequencies of their occurrence appeared in fish fed 10 000 μg U/g for 100 days.

Necrotic lesions, indicated by pyknotic hepatocyte nuclei, lysed cells, and other nuclear alterations, were frequent in the livers of treated lake whitefish (Figure 3.4B,D). Abnormal architecture was evident as disruptions of the normal cord-like arrangement of hepatocytes, such that hepatocytes were arranged irregularly or in rosette formations (Figure 3.4B).

Clear foci appeared as regions of liver parenchyma with little staining and in which hepatocytes were notably enlarged (Figure 3.4D). Affected cells typically contained 'flattened' nuclei that had been displaced by large vacuoles to the periphery of the cell; consistent with the description of excessive fatty accumulation provided by Peters et al. (1987). Based upon their rounded appearance, these vacuoles may have been lipids. However, because lipids are removed by the conventional histopathology techniques used here, the identity of the vacuole contents could not be confirmed.

The bile ductules of lake whitefish exposed to U were frequently damaged. Mild to moderate effects included notable lumina dilations (Figure 3.5B) and nuclear alterations, such as vacuolation (Figure 3.5C, Table 3.4). In the most severe instances, the bile ductule epithelial cells were necrotic as evidenced by the presence of pyknotic nuclei and epithelial desquamation (Figure 3.5D). Some ductules contained large amounts of debris, and in severe cases the lumina were occluded.

Semi-quantitative analyses of liver demonstrated the frequency and extent of necrotic and other degenerative lesions in this tissue of treated fish (Figure 3.6). Within each sampling day, the occurrence and the severity of necrosis was dose-dependent. Lesions included hepatocytes with pyknotic, karyorhectic, or otherwise morphologically

altered nuclei, hypertrophic cells, lysed cells, clear foci, and foci containing cell debris and nuclei. Hepatocytes with markedly dark-staining nuclei lacking clearly demarcated nuclear and cellular membranes were also common. In other instances, hepatocytes contained enlarged, notably pale-staining nuclei with disruptions in the nuclear membranes. In the most severe instances, voids in the liver parenchyma had formed, often containing nuclei and debris. Presumably these voids were a result of extensive hepatocyte death, as suggested by the presence of borders of lysed and necrotic hepatocytes. Necrotic regions commonly extended from bile ductules and veins but were also present in areas free of large blood vessels or biliary structures. Pigment deposits were occasionally present near lesions. Large lesions sometimes contained islands of regenerating hepatocytes, which were markedly eosinophilic and lacked cytoplasmic vacuoles (i.e. glycogen and lipids). Periodically, the livers of control fish contained small foci (i.e. a few cells) of degenerating hepatocytes.

Quantitative analyses revealed several changes in the morphometry of the liver parenchyma of treated lake whitefish relative to unexposed fish (Table 3.5). Fish fed the lowest concentration of U displayed only one alteration; a significant decrease in mean nuclear diameter on day 10 ($P < 0.100$) and day 100 ($P < 0.050$). In fish fed the moderate concentration of dietary U, a significantly increased relative hepatocyte size on days 30 and 100 ($P < 0.050$), decreased N:C on days 30 and 100 ($P < 0.050$), and decreased nuclear diameter on day 100 ($P < 0.050$) were observed. Fish from the highest treatment group displayed a transient reduction of hepatocyte nuclear size on day 10 ($P < 0.050$) and a decreased N:C on day 100 ($P < 0.100$).

Posterior Kidney

The posterior kidney of freshwater fish consists of nephrons randomly distributed amongst haematopoietic tissues. The basic structure of the freshwater teleost nephron and of control lake whitefish posterior kidney are illustrated in Figures 3.7 and 3.8A, respectively. The nephron consists of a glomerulus (G) or renal corpuscle, a short neck segment (N), proximal tubules subdivided into morphologically distinct first (P1) and second (P2) segments, distal tubules (DT), and collecting ducts (CD). Both proximal and distal tubules are surrounded by blood sinuses where resorbed substances re-enter the circulation (Yasutake and Wales 1983).

In the posterior kidneys of lake whitefish fed U, alterations were observed in all segments of the nephron, the interstitial tissues, the vascular system, and the immune system. The most consistently observed histopathologies were: proximal tubule necrosis (Figure 3.8B), foci of dilated tubules (Figure 3.8C), increased abundance of PM (Figure 3.8D), haemorrhaging, inflammation (Figure 3.8B), depletion of haematopoietic tissues (Figure 3.8B), and distal tubule lesions (Figure 3.9B). The numbers of fish with specific lesions are presented in Table 3.6.

The epithelium of affected tubules was frequently necrotic. However, where tubules were severely affected, the features used to distinguish between tubule segments were no longer applicable and the precise identity of the tubules could not be discerned. Because they lacked the connective tissue which surrounds collecting ducts, it can be stated that these structures were either proximal or distal tubules. Foci of dilated tubules

were frequent in treated fish and the fractions of fish affected in treatment groups were dose-dependent, particularly on day 100. These foci, illustrated in Figure 3.8C, were characterized by tubules with markedly dilated lumina which typically contained excessive quantities of eosinophilic debris.

Hyperplasia of PM's was observed at all sampling times but was most frequent and most severe on day 100, particularly in the low and moderate treatment groups (Figure 3.8D). Although PM's do not form discrete aggregations in salmonids (Agius 1985), groups of PM's were common in the posterior kidneys of treated fish, particularly by day 100 (Figure 3.8D), but not in unexposed controls. Furthermore, PM proliferation was most notable in regions of kidney that were markedly damaged.

Interstitial tissues exhibited peritubular inflammation, a very common, and frequently severe, histopathology in treated lake whitefish (Figure 3.8B). Haemorrhaging was frequent at all sampling times and severe (i.e. extensive) in fish sampled on day 100. Often the haemorrhaging occurred in the peritubular blood sinuses, and with accompanying reductions in the densities of haematopoietic tissues, the kidney appeared to have a massive blood sinus. In other instances, veins had ruptured creating large blood sinuses.

The most common distal tubule effects were epithelial nuclear alterations (Figure 3.9B) including nuclear membrane disruptions, hypertrophy, vacuolation, and displacement from the normally central location in the cytoplasm. Hyalinization of PI's (Figure 3.9A) was observed in a few treated fish and was extensive and severe in the 'nearly moribund' fish. The most overt alteration in mesonephric ducts was the presence

of excessive debris, including nuclei, in the lumen (Figure 3.9D). However, epithelial necrosis was occasionally observed.

Glomerular lesions, including necrosis, coagulation (Figure 3.10B), thrombi (Figure 3.10C), and 'hyalinization' (Figure 3.10D), occurred almost exclusively in fish fed the highest dietary concentration of U and were most frequent on day 30. Glomerular 'hyalinization' was evident as highly eosinophilic deposits within Bowman's space.

Semi-quantitative assessments of structural alterations to P2 and P1 segments revealed dose- and duration-dependencies (Figures 3.11 and 3.12). While the total proportion of damaged tubules in treated fish was similar across sampling times, an increasingly greater proportion of tubules in all treatment groups were moderately and severely damaged with increasing exposure period.

IV. Discussion

LSI

The LSI of lake whitefish exposed to U was largely unchanged over 100 days of exposure. The exception, occurring in fish fed 10 000 µg U/g food for 100 days, is considered a manifestation of tissue degeneration and indicates that in this group, the liver was overtly affected. Reductions of LSI and percentages of hepatic dry residues also occurred in rats, which had developed resistance to U, repeatedly injected with sub-lethal doses of U (Haven 1949). Furthermore, the LSI of rats receiving varying doses of U by intraperitoneal injections decreased in a dose-dependent manner (Maynard et al. 1953).

Haematology

U exposure caused few and only transient disturbances in the haematological profiles of lake whitefish. It was concluded in two reviews of contaminant effects on fish haematolgy that the usefulness of serum electrolyte alterations as biomarkers of exposure is limited (Folmar 1993, Mayer et al. 1992). The results of the present study concur and indicate that they would be of little value for purposes of U biomonitoring. The low sensitivity, and lack of exposure dose- and duration-dependencies, of the haematological profiles of lake whitefish is in agreement with the responses of fish to chronic contaminant exposure in a controlled environment and of mammals exposed to U (Maynard and Hodge 1949).

The occurrence of haematological alterations on day 30 coincides with peak accumulation of U in gills and gonads, the greatest degree of gonadal growth observed through the course of the exposure, the greatest frequencies of glomerular lesions in the high treatment group, and the highest observed concentrations of serum lipid peroxides. The concurrence of these observations indicates that secondary stress effects may arise, or be increased, when fish are simultaneously experiencing the demands associated with sexual maturation and U exposure. The ability of fish to cope with the individual stresses could be overwhelmed when exposed to both, resulting in a cascade of secondary health effects. Alternatively, the disappearance of haematological alterations by day 100, may be a consequence of the acquisition of resistance, or acclimation, to U.

Metallothionein

Metallothionein is an ubiquitous, low-molecular-weight (7 kDa), heat-stable, cysteine-rich, heat shock protein, whose structure is remarkably similar across taxa (Kille et al. 1992). Under non-pathological physiological conditions, the primary function of this protein is believed to be its participation in the maintenance of copper and zinc homeostasis (Olsson 1996). Because MT scavenges free radicals which may be generated in the presence of metals, MT also functions as an antioxidant (Hamer 1986, Matsubara et al. 1987).

MT synthesis is induced by trace amounts of non-essential metals Cd, Hg, and Ag, and excessive levels of Cu and Zn in fish (Roesijadi 1992, Wood et al. 1996). In turn, MT sequesters these metals, thus functioning as what is most often interpreted as a detoxification protein (Roesijadi 1992). Additionally, the metals Au, Pt, and Bi induce and bind to MT in mammals, whereas Ni and Co appear to be capable of MT induction but are not chelated by it (Garvey 1990, Kelley et al. 1988, Petering et al. 1990, Srivastava et al. 1995). Comparable MT-inducing abilities of these metals in fish are as yet unknown, although Pb failed to induce MT in cultured turbot fibroblasts (Kille et al. 1992).

MT induction in tissues of feral fish inhabiting metal-contaminated environments has received a great deal of attention and is advocated as a reliable biomarker of metal exposure (Deniseger et al. 1990, Garvey 1990, Klaverkamp et al. 1991, Petering et al. 1990). The use of MT as an indicator of metal exposure in fish and other aquatic organisms has been the subject of a number of recent reviews (Benson et al. 1990, Garvey

1990, Hogstrand and Haux 1991, Olsson 1996, Roesijadi 1992). Hence it is of interest to determine the ability of U to induce MT in fish.

Although dietary U induced MT in the liver of lake whitefish fed 10 000 μg U/g for 10 days, this response did not persist throughout the exposure period. Observations of acclimation to U in mammals suggest the involvement of MT induction (Balazs 1974, Barnett and Metcalf 1949, Haven 1949), because the characteristics of this resistance to U following chronic sub-lethal doses is similar to that which develops via MT induction. Furthermore, U exists predominantly or exclusively as the uranyl ion (UO_2^{2+}) in biological systems (Leggett 1989), and thus shares a chemical similarity, namely a divalent positive charge, with other MT-inducing metals.

There are a number of possible explanations for the disappearance of MT induction after day 10 in fish from the high treatment group: (1) the progressive decline in U dosage due to fish growth, (2) overt U toxicity, (3) reproductive influences, (4) the consumption of MT as an antioxidant, and/or (5) acclimation via other pathways and mechanisms. Further research is required to clarify the ability of U to induce MT in fish. A thorough discussion of these five explanations listed above is provided in chapter four.

No immediate explanation is apparent for the observed decrease of renal MT in fish fed 1000 μg/g for 100 days. This occurrence could be a manifestation of U toxicity, resulting from U-induced inhibition of protein synthesis (Ghadially et al. 1982), from significant renal damage, or from the consumption of MT as an antioxidant. However, because this effect was not seen in the highest treatment group where renal pathologies were most marked, this explanation does not seem adequate.

Lipid Peroxidation

Lipid peroxidation is a process in which the polyunsaturated fatty acids (PUFA) of phospholipid membranes are damaged by free radicals, such as superoxide ($O_2^-\cdot$) and hydroxyl (OH•) radicals, and oxygen (Di Giulio 1991). The reaction, which is capable of self-propagation, involves the abstraction of a hydrogen atom and terminates in the formation of a lipid hydroperoxide, one of the endpoints of oxidative stress. Because basal levels of lipid peroxidation occur in all tissues under normal physiological conditions, organisms are constantly combatting oxidative damage. However, stress that directly or indirectly enhances the production of free radicals or reduces the antioxidant defence systems of a tissue will expedite the process.

Fish are particularly vulnerable to lipid peroxidation owing to the presence of a high fraction of unsaturated relative to saturated fatty acids in their membrane systems (Agius 1985). Higher fractions of PUFA, fatty acids which are fluid at low temperatures, in fish allow the maintenance of membrane fluidity at low body temperatures, a characteristic which is viewed as an ectothermic adaptation (Agius 1985). Recent comparisons of the effects of Cu and Hg on erythrocyte haemolysis and antioxidant enzyme activities in fish and humans demonstrated that the former are considerably more sensitive to oxidative stress (Gwozdzinski et al. 1992).

U caused a dose-dependent increase in the concentration of lipid peroxides in lake whitefish serum on days 30 and 100. However, higher concentrations were observed in all three treatment groups on day 30. Lipid peroxidation is biologically relevant because

it compromises the structural and functional integrity of biological membranes by increasing membrane structural order and damaging membrane proteins and enzymes (Horton and Fairhurst 1987). Ultimately, lipid peroxidation may cause cell death and tissue damage (Halliwell and Gutteridge 1985, Horton and Fairhurst 1987).

Indices of oxidative stress are attractive biomarkers because they provide valuable information regarding contaminant-induced stress and are of immediate significance to organismal health. Oxidative damage has been implicated in a number of human diseases and etiologies, such as: cancer, mutation, aging, vascular diseases, tissue necrosis, Parkinson's disease, cataracts, multiple sclerosis and other neurological pathologies, autoimmune disorders, and inflammation (Kehrer 1993, Sahu 1991, Suzuki et al. 1995), and is suspect in the development of similar effects in fish (Winston and Di Giulio 1991). Because at least two of the aforementioned pathologies, tissue necrosis and inflammation, were observed in lake whitefish in this study, lipid peroxidation may be one of the molecular mechanisms by which histopathological lesions arose. This possibility warrants further research.

There are several possible explanations for the occurrence of higher concentrations of LPO on day 30 relative to day 100, including: (1) decreasing U dosage with increasing time, (2) U acclimation, and (3) gonadal maturation. The first explanation may be adequate if a critical concentration of U were required for MT induction to occur. Because the experimental lake whitefish grew throughout the course of this study, yet the feeding ration and concentration of U in fish food remained constant, fish were exposed to progressively lower doses of U with increasing exposure duration.

Alternatively, fish may have acclimated to U. Acclimation is a characteristic response in mammals exposed to U by various routes for prolonged periods. Species for which this has been demonstrated are dogs, rats, and rabbits (Haven 1949, Hodge 1973, Stopps and Todd 1982, Yuile 1973). There is also circumstantial evidence that humans develop resistance to U, based upon observations of patients who received U as a treatment for diabetes mellitus during the late 1800's (Stopps and Todd 1982). That teleosts also possess the ability to acclimate to U could explain temporal reductions in toxicity responses that were observed in fish from this study.

The third explanation is plausible because developing gonads contain a high fraction of PUFA (Steffens 1989) and, thus, are particularly vulnerable to lipid peroxidation. Hence peak concentrations of LPO on day 30 could have arisen from enhanced oxidative degradation of this tissue. Alternatively, the consumption of vitamin C and E reserves by maturing gonads could have indirectly caused enhanced lipid peroxidation in other soft tissues by reducing their antioxidant capacities. These vitamins protect both male and female gonads from lipid peroxidation, due to their antioxidant properties, and ultimately improve sperm, egg, and embryonic viabilities (Liu et al. 1997, Saborowski et al. 1997, Steffens 1989, Watanabe 1985). There is evidence that ascorbic acid is shunted from other soft tissues to meet the increased demands in the maturing gonads of fish (Saborowski et al. 1997). Depletions of antioxidants in these tissues could increase their vulnerability to lipid peroxidation. A more thorough discussion of the role of gonadal maturation in lipid peroxidation is provided in chapter four.

The ability of metals other than U to increase lipid peroxidation in fish has been the focus of intensive study in recent years. Increases in indices of lipid peroxidation in various tissues of feral fish inhabiting metal-enriched systems have been reported (Farag et al. 1994, 1995, Woodward et al. 1995). Metals which increase lipid peroxidation in marine and freshwater fish in the laboratory are: Cd (Palace et al. 1993), Cu (Gwozdzinski et al. 1992, Radi and Matkovics 1988), Fe (Baker et al. 1997), Hg (Gwozdzinski et al. 1992), Pb (Arias et al. 1991, Chaurasia et al. 1996), and V (Chakraborty et al. 1995).

The analysis of lipid peroxides in fish tissues proved one of the most sensitive and reliable indicators of U exposure and toxicity in lake whitefish. Assessments of oxidative stress parameters have received a great deal of attention in aquatic toxicology and have been advocated as contaminant biomarkers in the aquatic environment (Bainy et al. 1996, Di Giulio et al. 1989, Holdway et al. 1995, Jimenez and Stegeman 1990, Livingstone 1993, Thomas 1990). The results of this study support these advocations.

The present results only equivocally demonstrate that U enhances lipid peroxidation. Further research is required to confirm the direct involvement of free radicals in U toxicity. Several researchers have emphasized that increased LPO do not empirically demonstrate cause and effect, because lipid peroxidation may increase secondarily to other pathologies, without any involvement of free radicals (Di Giulio et al. 1989, Kehrer 1993, Shaheen et al. 1996). This lack of mechanistic information on U toxicity, however, does not detract from the potential of adopting serum LPO as sensitive biomarkers of U exposure and toxicity in fish.

Histopathology

Liver

Histological alterations in the livers of fish are frequently observed following exposure to a variety of contaminants in the laboratory and in the natural environment. Thorough discussions of the use of liver histopathologies in fish as bioindicators of contaminant toxicity are provided by Hinton and Lauren (1990a and 1990b) and Hinton et al. (1992).

Numerous histopathologies were present in the livers of lake whitefish exposed to U, all of which have previously been observed in fish exposed to contaminants in the lab and/or in the natural environment. Reports of lesions similar to those observed in lake whitefish include: abnormal liver architecture (Jha et al. 1994, McCain et al. 1977, Peters et al. 1987, Sorensen et al. 1980), focal fatty vacuolation of hepatocytes or 'clear foci' (Cormier and Racine 1990, Hinton and Lauren 1990a, McCain et al. 1977, Peters et al. 1987, Sorensen et al. 1984, Teh et al. 1997, Vethaak and Wester 1996), alterations, such as hydropic vacuolation and membrane damage, of biliary epithelium (Hinton and Lauren 1990a, Myers et al. 1994, Vethaak and Wester 1996), and parenchyma necrosis (Adams et al. 1990, Cormier and Racine 1990, Hinton et al. 1973, Hinton et al. 1988, McCain et al. 1977, Myers et al. 1994, Peters et al. 1987, Vethaak and Wester 1996). Hinton and Lauren (1990a) concluded that biliary epithelial alterations are meaningful biomarkers of

contaminant exposure and that necrotic lesions in liver are one of the few validated histopathological biomarkers of contaminant exposure in fish.

Necrosis in the liver of lake whitefish exposed to U could result from hepatocyte lysosomal rupture and subsequent tissue autolysis. U accumulates in lysosomes of marine and freshwater molluscs and crustaceans (Chassard-Bouchaud 1982, 1983, Chassard-Bouchaud and Hallegot 1984) and mammals (Galle 1974, Galle et al. 1992, Muller et al. 1989, Tasat and de Rey 1987), and can pierce the membrane when precipitated as a U phosphate microneedle crystal (mammals: Galle 1974). Lysosomal membrane damage and the subsequent release of hydrolytic enzymes into the cytosol may have been the mechanism of severe damage to organelles and cell death observed in rat pulmonary macrophages treated with U (Tasat and de Rey 1987). Furthermore, cells with damaged lysosomal and cellular membranes could leak these hydrolytic enzymes into extracellular fluids where they could, in turn, damage other cells (Tasat and de Rey 1987).

Conversely, necrosis could be caused by tissue hypoxia, a condition that often arises in fish exposed to contaminants (Hinton and Lauren 1990a). Because U has a particular affinity for mitochondria (Stevens et al. 1980) where it has been found to disrupt the functional and structural integrity of this organelle (Carafoli et al. 1971, Leggett 1989), it is conceivable that cellular respiration may have been impaired. Alternatively, hepatocyte necrosis could be the result of enhanced lipid peroxidation and subsequent membrane damage (Kehrer 1993). Consideration of this potential mechanism of cellular injury is attractive, owing to the extensive literature on mammals which

indicates U is a potent membrane toxin (e.g. Carafoli et al. 1971, Leggett 1989, Muller et al. 1989).

Clear cell foci observed in the livers of treated lake whitefish may be sites of excessive fat storage. Typically, hepatocytes contain lipid and glycogen deposits. In fish supplied with adequate food, the cytoplasm appears mottled in histological preparations because these substances are leached in aqueous fixatives and conventional tissue processing techniques and the remaining vacuoles are not stained by the H&E method (Peters et al. 1987, Simon et al. 1967). It is likely, however, that clear cell foci were lesions of fatty degeneration of hepatocytes because the livers of treated fish were frequently whitened and mottled macroscopically; a gross appearance characteristic of this histopathology (Hinton et al. 1973, Hinton and Lauren 1990a, b, Roberts 1978). Furthermore, the appearance of affected cells was similar to descriptions of lesions of lipid degeneration in the livers of feral fish reported in the literature (Peters et al. 1987).

Significant alterations of hepatocyte morphometrics were observed in all groups of fish fed contaminated diets. In the highest treatment group, hepatocyte nuclei were reduced in size early in the course of the exposure but recovered by day 30. In fish consuming low and moderate U concentrations, reduced nuclear diameters were pronounced later in the exposure period (d 100), concomitant with increased relative hepatocyte size and a subsequent reduced N:C ratio in the latter group. However, it is not known whether these alterations would persist in fish from these two groups following prolonged exposure. It would appear that alterations in hepatocyte morphometrics are dependent upon dose and duration of exposure, and, may not persist. Reductions in the

N:C ratios in the livers of fish from the highest treatment group on day 100, may indicate the onset of chronic alterations. This response has been observed in hepatocytes that survived the initial stages of contaminant-induced cytotoxicity in fish (Hinton et al. 1988). Due to the variability of the present data, the morphometry of liver cells were not reliable indicators of U exposure. To ascertain the usefulness of these parameters in field applications, it is necessary to examine them further in fish exposed to U for longer durations.

Alterations in the morphometry of hepatocytes have been observed in fish exposed to contaminants and some of the mechanisms of these changes have been elucidated. Reductions in the sizes of nuclei may be early morphological manifestations of cytotoxicity that develops during chronic exposure to toxins (Peters et al. 1987). Increased relative hepatocyte size and reductions in N:C may arise from excessive lipid and/or glycogen reserves, from hydropic degeneration (i.e. cell swelling) caused by denaturation of ATPases or alterations of the cellular energy transfer processes, or from organelle proliferation, often endoplasmic reticulum (Hinton and Lauren 1990a, b). All of these causes of hepatocyte enlargement are established responses in fish chronically exposed to contaminants, although the latter is the most common (Hinton and Lauren 1990a, b). A reduction in the N:C ratio may also be indicative of the early stages of neoplasia, because this characteristic is typical of undifferentiated, rapidly growing cells (Hinton et al. 1988). However, the decreased N:C observed in fish from the moderate treatment group is at least partly a result of concomitant reductions in nuclear, and increases in relative hepatocyte, sizes.

The occurrence of hepatocyte and biliary histopathologies in lake whitefish exposed to dietary U suggests that biliary excretion of U is a significant depuration pathway. This is consistent with the prominent role of the bile in the excretion of other metals orally administered to fish (Handy 1996, Lanno et al. 1987, Weisbart 1973). Liver damage in mammals exposed to U appears to be less common and of lesser significance than that seen in lake whitefish. However, where mammals are exposed to U in the diet, liver injury is more frequent than following other routes of U administration (Barnett and Metcalf 1949). High single doses of U ingested by mammals, including humans, impair liver functioning (e.g. Pavlakis et al. 1996).

Posterior Kidney

Numerous histopathologies were evident in the posterior kidneys of lake whitefish consuming U. This is consistent with the extensive literature on mammals, which indicates that the primary target tissue for U is the kidney and that U exposure by any route causes a host of renal histopathologies (Leggett 1989, Voegtlin and Hodge 1949, 1953). In the most severe cases, U intoxication proves fatal due to the development of acute renal failure (e.g. Ubios et al. 1994). Generally, U damages the proximal tubules resulting in necrosis, desquamation, dilation, and the accumulation of tubule debris (Barnett and Metcalf 1949, Domingo et al. 1987, Griswold and McIntosh 1973). These pathologies were frequent in lake whitefish fed contaminated diets. With prolonged exposure, tubules are regenerated and substantial recovery of renal structure and functioning may occur in mammals (Barnett and Metcalf 1949).

Lesions of the proximal tubules of lake whitefish may have arisen via mechanisms similar to those which operate in mammals. As discussed in chapter two, in mammals U-carbonate complexes, which are filtered across the glomerulus, dissociate in the acidic environment of proximal tubule lumens, thus freeing U to interact with the brush borders of epithelial cells (Dounce 1949). U has a high affinity for anionic sites on the lumen membrane of proximal tubule epithelium, including the side groups of structural proteins and phospholipids, and it is through this binding that U is believed to exert some of its toxicity (Leggett 1989, Pasquale et al. 1986). Accumulation of U in lysosomes of the tubule epithelium and subsequent lysosomal rupture may damage organelles and cellular constituents, as suggested for liver (Galle 1974, Tasat and de Rey 1987). Other organelles for which U demonstrates an affinity in mammals are rough endoplasmic reticulum, nuclei, and particularly mitochondria (Leggett 1989, Stevens et al. 1980). In turn, U disrupts mitochondrial structure and function (Carafoli et al. 1971, Leggett 1989), impairs protein synthesis (Ghadially et al. 1982), and is genotoxic (Au et al. 1995, Lin et al. 1993), all of which could manifest as histopathologies.

The various glomerular lesions including, hyalinization, thrombi, and necrosis, observed in lake whitefish, are consistent with mammalian data. Glomerular damage is common in mammals exposed to U, although the precise mechanisms of this toxicity are unresolved. Necrotic glomeruli and hyalin deposits in glomeruli and proximal tubules have been observed in mammals exposed to this nephrotoxin (Barnett and Metcalf 1949, Domingo et al. 1987). However, the glomerulus has received less attention by histopathologists than the proximal tubules (Stopps and Todd 1982).

In the present case, however, the composition of glomerular deposits, commonly referred to as 'hyalin', can not be precisely identified at the level of resolution afforded by light microscopy. Thus, this pathology may have resulted from accumulation of amyloid, an abnormal synthetic product of cells, or hyalin in lake whitefish (Francis 1990). Glomerular hyalinization is an endpoint of chronic renal failure in mammals, arising from reductions in the glomerular filtration rate (Stevens and Lowe 1992). P1 hyalinization, which occurred in a few individual fish, is also an overt indicator of glomerular damage. These eosinophilic cytosolic deposits are accumulations of excessive quantities of resorbing proteins that were filtered across damaged glomeruli, and hence are manifestations of proteinuria (Hinton and Lauren 1990b).

That glomerular lesions were most frequent in fish fed 10 000 µg U/g food for 30 days is consistent with other effects observed at this time. However, because glomerular lesions were seen almost exclusively in fish from the highest treatment group, they do not appear to be sensitive indicators of U exposure.

Glomerular lesions could prove more reliable indicators of U exposure using more sensitive techniques, such as electron microscopy (EM). At the level of resolution offered by light microscopy, only overt alterations of glomeruli can be discerned. Hence, more covert histopathologies would have gone unnoticed in the present work. Using EM, glomerular lesions were demonstrated in rabbits exposed to U in drinking water. Lesions included dose-dependent thickening and budding of glomerular basement membranes (GBM), an effect which persisted throughout recovery periods. Alterations of the GBM may have arisen from mesangium damage, loss of membrane integrity and increasing

cellular rigidity, or the deposition of immune complexes (McDonald-Taylor et al. 1992). Morphologic alterations of GBM were also observed in rats exposed to U (Griswold and McIntosh 1973).

Other histopathologies, including alterations of distal tubules and mesonephric ducts, inflammation, and haemorrhaging, induced by dietary U in the posterior kidney of lake whitefish have also been reported in U-treated mammals. Damage to segments of the nephron beyond the proximal tubules has been observed in mammals exposed to U (Barnett and Metcalf 1949, Bowman and Foulkes 1970). Inflammation (nephritis) is a characteristic response to chronic U challenge in mammals (Barnett and Metcalf 1949, Hursh and Spoor 1973). Microhaemorrhagic foci were observed in rats administered uranyl acetate dihydrate subcutaneously (Domingo et al. 1987).

Hyperplasia of PM in lake whitefish posterior kidneys may indicate enhanced tissue degeneration, because the primary functions of these cells are believed to be detoxification and phagocytosis of tissue debris and foreign substances (Secombes and Fletcher 1992, Wolke 1992). The occurrence of aggregations of macrophages in haematopoietic tissues and liver is one of the characteristics of the teleost immune system. Typically these groupings, referred to by a number of terms: melano-macrophage centres (Roberts 1975), macrophage aggregations (Blazer et al. 1987, Wolke et al. 1985), macrophage centres (Haaparanta et al. 1996), pigment nodules (Fulop and McMillan 1984), and most recently, pigmented macrophage aggregates (Couillard and Hodson 1996), consist of macrophages containing any or all of four primary pigments haemosiderin, melanin, lipofuscin, and ceroid. In addition, various lymphoid elements,

such as lymphocytes, associate with pigmented macrophages, together forming a fundamental component of the immune system (Agius 1985).

Unlike most teleosts, however, salmonids do not form discrete and organized aggregations of PM (Agius 1985) and thus PM occur primarily as individual cells or in small disorganized groupings in the kidneys of lake whitefish. Fish chronically exposed to heat or contaminant stress may contain a greater number of, an increased density of, and/or larger PMA. Hence, a number of researchers have explored this phenomenon as a biomarker of contaminant exposure in fish (Blazer et al. 1987, Couillard and Hodson 1996, Haaparanta et al. 1996, Teh et al. 1997, Vethaak and Wester 1996, Wolke et al., 1985).

PM proliferation was most notable in fish fed low and moderate concentrations of U for 100 days. That fish from the highest treatment group sampled on day 100 exhibited a lower frequency of this response, and to a lesser degree in individual fish, could be explained by overt toxicity. Flounder, ruffe, and smelt, inhabiting the Lower Elbe, heavily contaminated with metals, typically contained larger and more numerous PMA in livers with significant degenerative lesions (Peters et al. 1987). However, where 'especially serious degenerative changes' were present, PMA were reduced in size and number. This reduction was assumed to be a consequence of contaminant toxicity in these centres.

Overt toxicity to fish macrophages could arise where contaminants concentrate in these cells. Contaminants contained in degrading tissue, once phagocytosed, may accumulate in PM. Melanin, one of the main pigments, binds numerous contaminants

(Agius 1985). Accumulation of contaminants in teleost PMA is analogous to contaminant accumulation in the lymph nodes of mammals. The greatest accumulation of U in occupationally exposed humans (i.e. U mill workers) was found in this tissue (Singh et al. 1987a). Furthermore, U accumulates in rat alveolar macrophages in lysosomes and phagosomes (Muller et al. 1989, Tasat and de Rey 1987) or in the cytosol in association with ferritin (Muller et al. 1989). *In vitro* treatment of macrophages with aqueous U caused marked ultrastructural changes and cell death (Muller et al. 1989). If U has a similar effect on the renal macrophages of fish, this contaminant could potentially be immunosuppressive and predispose organisms to disease.

Kidney histopathologies are currently considered promising candidates as contaminant biomarkers in fish (Hinton et al. 1992). Additional supporting research is required for this tissue. Renal lesions are not yet established biomarkers due to the inadequate volume of histopathological research and the lack of corroborating results of field and laboratory studies. However, all of the posterior kidney histopathologies observed in the present study have been reported in fish exposed to contaminants in controlled environments and/or in the field (Bucke and Feist 1985, Hall et al. 1987, Hicks et al. 1984, Hinton et al. 1973, 1992, Kumar and Pant 1985, Myers et al. 1994, Schwaiger et al. 1997, Sorensen et al. 1984, Tafanelli and Summerfelt 1975, Trump et al. 1975, Walsh and Ribelin 1975, Wester et al. 1988, Wobeser 1975). Hence, it is noted that, like many contaminant-induced lesions, the renal histopathologies observed in lake whitefish are not unique and thus not specific to U.

The most appropriate renal histopathological indicators for U appear to be: haemorrhaging, inflammation, depletion of haematopoietic tissues, proximal tubule necrosis, focal dilation of tubules, and distal tubule lesions. Proliferation of PM was characteristic in fish fed low and moderate concentrations of U and may prove to be a useful indicator in feral fish experiencing more prolonged exposure. Of the types of assessments employed for histopathological analysis, qualitative assessments are considered the most appropriate as they yielded the greatest degree of information and facilitated the inclusion of lesions that are not readily quantifiable.

V. Summary and Recommendations

The consumption of a diet containing U by lake whitefish caused a number of deleterious effects, which were evaluated at several levels of biological organization. Parameters at the higher levels of biological organization (wet weight, fork length, and CF) were unaffected by U and may not be useful indicators of exposure and subsequent health effects in feral fish inhabiting contaminated aquatic systems. Indices of toxicity evaluated at the tissue level proved more sensitive and reliable as biomarkers of exposure to U in lake whitefish. Haematological variables and LSI were largely unaltered in treated fish and, although more sensitive indicators than whole organism parameters, are not the most promising candidates for environmental monitoring of fish. Histopathological evaluations of liver and posterior kidney from treated fish revealed numerous lesions. By the final sampling day (d 100) histopathologies were evident in all

fish consuming contaminated diets. Of the two tissues, the kidney was more extensively affected. The inclusion of histopathological analyses of liver and posterior kidney of fish inhabiting systems impacted by U enrichment is strongly advocated. This type of assessment is viewed as valuable due to its ability to reveal U exposure and due to its immediate relevance in terms of assessing fish health.

The biochemical parameters investigated in the present study, liver and kidney MT and serum lipid peroxides, were differentially affected by U treatment. In the case of the former, little effect of U exposure was found. However, because fish underwent gonadal maturation during the course of the study these results are viewed as inconclusive and further investigations into the ability of U to induce MT are needed. Conversely, the concentration of LPO in lake whitefish serum proved a sensitive and reliable indicator of U exposure. Strong dose-dependent increases in serum LPO were observed on days 30 and 100. Higher concentrations of serum LPO were seen on day 30 in all groups of fish treated with U. The most likely explanation for this anomaly is the concurrent growth of lake whitefish gonads, which may render the fish more sensitive to U during this critical and demanding physiological period. This suggestion requires further evaluation. However, without empirical data to support the latter contention, the possibility that fish acclimated to U following chronic exposure remains valid.

Collectively, the results of the assessment of U toxicity in lake whitefish consuming contaminated diets suggest that U is toxic at doses at least as low as 100 µg U/g food administered at a relatively low feeding ration. Because this concentration falls within the lower end of concentrations of U found in the sediments of aquatic ecosystems

impacted by U mining and milling, this is of environmental significance. Assessments of fish health which do not incorporate tissue and biochemical indices of toxicity may fail to reveal adverse effects attributable to U in feral fish inhabiting these systems. This argues strongly for the use of a suite of bioindicators encompassing several levels of biological organization in evaluations of the health of feral fish; an approach that is often advocated by contemporary aquatic toxicologists (Adams et al. 1990, 1996, Depledge et al. 1995, Holdway et al. 1995, Livingstone 1993).

Furthermore, the present findings question the present prioritization of concern and emphasis placed upon contaminants other than U in aquatic environments receiving U mining and milling discharges. In turn, it is advised that greater attention be devoted to U in these systems, as was concluded by Hynes (1990) in his assessment of lakes in the vicinity of the Cluff Lake mine and mill, Saskatchewan. Biomarkers of U exposure and toxicity that could be incorporated into assessments of feral fish health are liver and kidney histopathology and indices of oxidative stress.

Future research could include further evaluations of the sites and mechanisms of lipid peroxidation, evaluations of other endpoints of oxidative stress, such as genotoxicity, validation of the ability of U to induce MT or other stress proteins (i.e. heat shock proteins), as has been suggested by mammalian researchers (Furuya et al. 1997, Mizuno et al. 1997), and assessments of the effects of U on the structural integrity of the major sites of accumulation, bone and scales. It would also be of great value to characterize the interactions between U toxicity and fish reproduction, including the

identification of potential adverse effects of U on embryonic development and offspring

viability.

Table 3.1. Lake whitefish sex ratios and morphometrics wet weight, fork length, and condition factor, on days 10, 30, and 100 of exposure to a diet contaminated with uranium. Data are expressed as mean ($\pm$ SE). Asterisks denote values significantly different from controls (P < 0.05).

Treatment (µg U/g)	Sex Ratio (Females : Males)			Wet Weight (g)			Fork Length (cm)			Condition Factor (CF)		
	d 10	d 30	d 100	d 10	d 30	d 100	d 10	d 30	d 100	d 10	d 30	d 100
0	4 : 2	3 : 3	2 : 4	683 (48)	635 (40)	786 (58)	35.8 (0.5)	34.6 (0.9)	37.1 (0.3)	1.48 (0.07)	1.54 (0.05)	1.54 (0.10)
100	2 : 4	5 : 1	2 : 4	663 (26)	733 (48)	731 (37)	37.6 (2.2)	36.0 (0.9)	37.0 (0.5)	1.32 (0.14)	1.56 (0.03)	1.44 (0.04)
1000	4 : 2	5 : 1	3 : 3	681 (48)	706 (31)	753 (85)	36.4 (1.0)	36.2 (0.7)	36.9 (1.4)	1.41 (0.04)	1.49 (0.03)	1.48 (0.05)
10000	4 : 2	2 : 3	2 : 4	663 (80)	644 (90)	726 (76)	35.7 (1.4)	34.8 (1.4)	36.3 (1.2)	1.43 (0.06)	1.48 (0.05)	1.49 (0.05)

Table 3.2. Haematological parameters, haematocrit, serum K^+, Na^+, and Cl^-, serum osmolality, and the fractional contribution of Na^+ and Cl^- to osmolality ($Na^+ + Cl^-$ / osmolality) of lake whitefish fed an uncontaminated and a U-contaminated diet for 10, 30, and 100 days. Data are expressed as mean ($\pm$ SE). Asterisks denote values significantly different from controls ($P < 0.05$).

	Day 10 Diet (μg U/g)				Day 30 Diet (μg U/g)				Day 100 Diet (μg U/g)			
	0	100	1000	10 000	0	100	1000	10 000	0	100	1000	10 000
Haematocrit (%)	40.9 (0.5)	43.8 (2.1)	40.9 (0.7)	39.5 (2.2)	43.3 (1.5)	41.8 (3.5)	40.6 (1.1)	39.7 (1.5)	36.8 (1.8)	34.6 (4.9)	38.3 (1.4)	37.5 (3.3)
K+ (mM/L)	2.59 (0.24)	3.01 (0.21)	2.69 (0.16)	2.75 (0.23)	2.84 (0.20)	2.93 (0.15)	3.24 (0.30)	3.15 (0.20)	3.16 (0.27)	3.12 (0.10)	2.58 (0.15)	2.84 (0.13)
Na+ (mM/L)	154.7 (0.8)	154.5 (0.4)	153.9 (0.5)	148.6 (3.5)	156.0 (0.8)	154.3 (0.7)	154.9 (0.7)	154.8 (0.7)	152.2 (0.5)	150.5 (1.2)	151.7 (0.4)	152.3 (1.0)
Cl- (meq/L)	133 (1)	133 (1)	133 (1)	127 (7)	130 (1)	131 (1)	135* (1)	136* (1)	134 (1)	131 (1)	136 (1)	136 (1)
Osmolality (mOsm/L)	318 (2)	317 (1)	318 (2)	313 (8)	319 (4)	319 (3)	325 (1)	329* (2)	314 (1)	310 (3)	317 (1)	316 (1)
Na + Cl / Osmolality (%)	90.4 (1.0)	90.8 (0.6)	90.2 (0.2)	88.0 (1.2)	89.8 (1.3)	89.2 (0.7)	89.3 (0.5)	88.4 (0.5)	90.9 (0.7)	90.7 (0.6)	90.8 (0.3)	91.1 (0.5)

Table 3.3. Metallothionein concentrations in liver and kidney of lake whitefish fed an uncontaminated diet and diets contaminated with uranium for 10, 30, and 100 days. Data are expressed as mean (± SE). Asterisks denote values significantly different from controls (P < 0.05).

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Treatment (µg U/g food)	Liver MT (µg/g ww)			Kidney MT (µg/g ww)		
	d 10	d 30	d 100	d 10	d 30	d 100
0	307 (66)	372 (65)	383 (114)	13.9 (1.2)	15.9 (1.9)	23.0 (3.4)
100	402 (78)	346 (38)	458 (97)	12.2 (1.5)	15.3 (1.3)	17.6 (1.5)
1000	332 (85)	381 (68)	337 (88)	11.1 (1.0)	14.2 (3.0)	12.8* (1.2)
10 000	530* (59)	294 (74)	345 (88)	11.8 (3.0)	10.2 (1.7)	17.2 (2.2)

Table 3.4. Qualitative histopathological evaluations of the liver of lake whitefish fed an uncontaminated diet and diets contaminated with U for 10, 30, and 100 days. Data are expressed as the number of fish from each treatment group exhibiting the various lesions.

Tissue	Lesion	Day 10 Diet (μg U/g)				Day 30 Diet (μg U/g)				Day 100 Diet (μg U/g)			
		0	100	1000	10 000	0	100	1000	10 000	0	100	1000	10 000
n		6	6	6	6	6	6	6	5	6	6	6	6
Liver Parenchyma													
	Abnormal Archictecture	0	3	0	1	0	2	2	2	0	2	1	2
	Clear Cell Foci	0	2	0	1	0	0	1	2	0	2	0	3
Bile Ductules													
	Epithelial Cell Hypertrophy	0	2	0	1	0	1	0	0	0	0	0	1
	Epithelial Necrosis	0	2	6	3	0	3	4	5	0	6	6	6
	Lumina Debris	0	2	1	2	0	1	0	0	0	1	1	0

Table 3.5. Hepatocyte morphometrics in lake whitefish fed an uncontaminated and U-contaminated diets for 10, 30, and 100 days. Relative hepatocyte area, hepatocyte nuclear diameter, and nuclear area : cytoplasmic area ratio are presented as mean (± SE). Asterisks denote means significantly different from controls (**$P < 0.05$, *$P < 0.100$).

Day	Treatment (μg U/g)	n	Relative Hepatocyte Area[†] (μm^2)	Hepatocyte Nuclear Diameter (μm)	Nuclear Area: Cytoplasmic Area (%)
10	0	6	176 (12)	5.53 (0.07)	16.4 (1.4)
	100	6	154 (12)	5.23* (0.08)	16.9 (1.7)
	1000	6	173 (15)	5.27 (0.09)	15.2 (1.6)
	10 000	6	161 (22)	4.61** (0.11)	12.4 (1.3)
30	0	6	162 (7)	5.35 (0.10)	16.3 (1.0)
	100	6	156 (14)	5.35 (0.11)	17.5 (1.2)
	1000	6	212** (20)	5.19 (0.11)	11.6** (1.0)
	10 000	5	159 (15)	5.22 (0.16)	16.0 (1.4)
100	0	6	149 (8)	5.44 (0.06)	18.8 (1.3)
	100	6	153 (18)	4.92** (0.05)	15.3 (1.7)
	1000	6	205** (11)	4.95** (0.08)	10.6** (0.8)
	10 000	6	185 (15)	5.32 (0.12)	14.3* (1.4)

† One Way ANOVA on log-transformed data and a one-tailed Dunnett's multiple comparison

Table 3.6. Number of individuals exhibiting specific lesions in the posterior kidney of lake whitefish fed an uncontaminated diet and a diet contaminated with 100, 1000, and 10 000 µg U/g for 10, 30, and 100 days. Data are expressed as the number of fish from each treatment group exhibiting the various lesions. Haematopoietic tissue (HT), pigmented macrophage (PM), distal tubule (DT), mesonephric duct (MD).

	Day 10				Day 30				Day 100			
Lesion	Diet (µg U/g)				Diet (µg U/g)				Diet (µg U/g)			
	0	100	1000	10 000	0	100	1000	10 000	0	100	1000	10 000
n	6	6	6	6	6	6	6	5	6	6	6	6
Haemorrhaging	0	4	3	6	0	3	5	4	0	5	5	4
Inflammation	0	4	6	6	0	5	6	5	0	6	6	6
HT Depletion	0	6	6	6	0	4	5	5	0	6	6	6
PM Proliferation	0	3	4	3	0	3	5	2	0	6	6	4
Dilated Tubules	0	2	0	3	0	2	4	3	0	1	2	4
DT Lesions	0	5	5	3	0	6	5	5	0	6	4	6
MD Lesions	0	0	2	0	0	1	0	0	0	1	2	3
Proximal Tubules												
Necrosis	0	3	2	4	0	5	4	5	0	6	6	6
Hyaline Deposits	0	1	0	1	0	0	0	2	0	0	0	0
Glomeruli												
Dilated Capillaries	0	0	0	0	0	0	0	1	0	0	0	1
Necrosis	0	0	0	1	0	0	0	2	0	0	0	1
Hyalinization	0	1	0	1	0	0	1	3	0	0	1	1
Thrombi	0	0	0	0	0	0	0	1	0	0	0	1

Figure 3.1. The liver somatic indices (LSI) of lake whitefish fed an uncontaminated diet and a diet contaminated with 100, 1000, and 10 000 µg U/g for 10, 30, and 100 days. Data are expressed as mean (± SE). Asterisks denote means significantly different from controls sampled on the same day (P < 0.05).
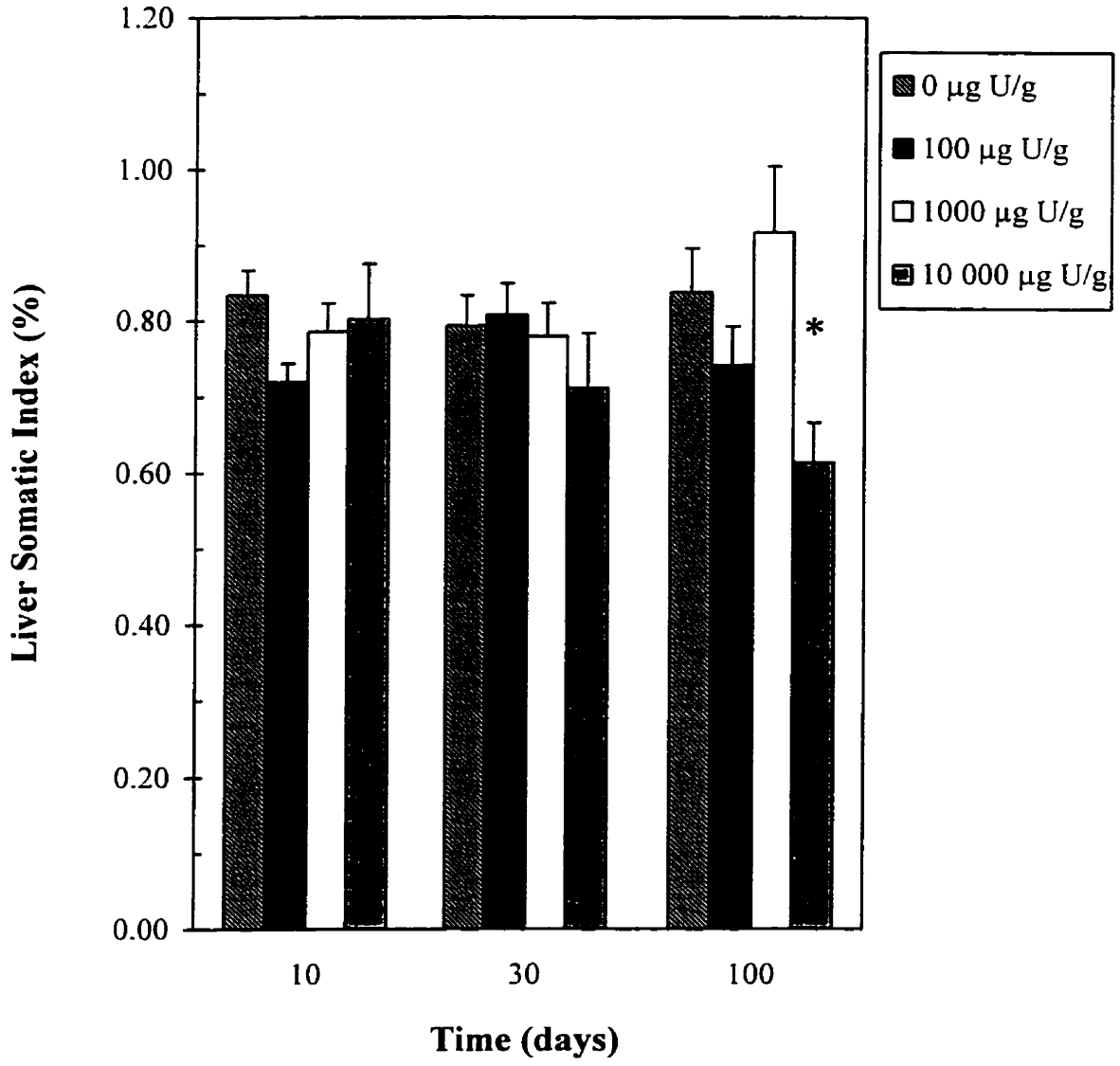
Figure 3.2. Concentrations of lipid peroxides in the serum of lake whitefish fed and uncontaminated diet and a diet contaminated with 100, 1000, and 10 000 µg U/g for 10, 30, and 100 days. Data are expressed as mean (± SE). Asterisks denote means significantly different from controls sampled on the same day (P < 0.05).
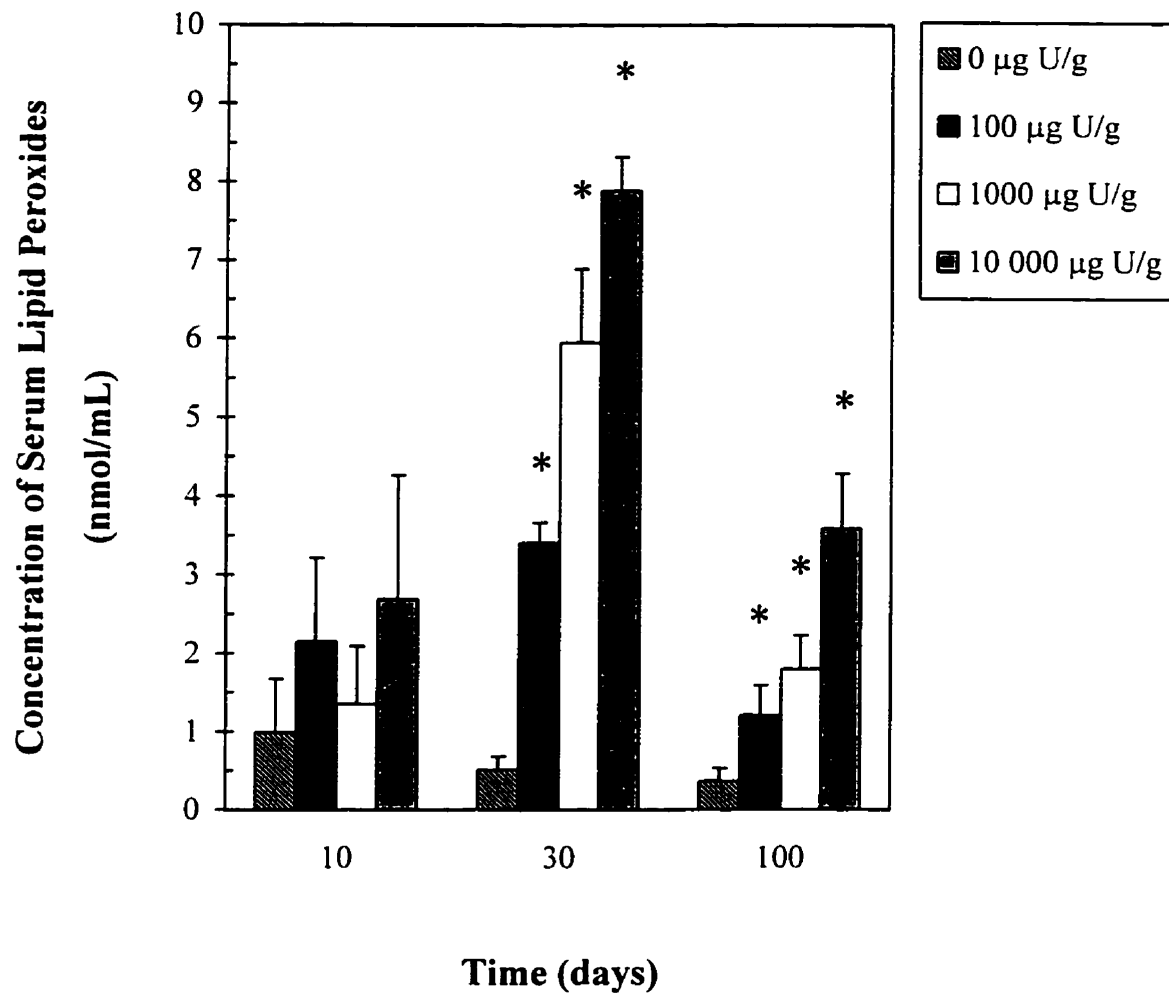
Figure 3.3. The histological appearance of the liver of lake whitefish fed an uncontaminated diet (A & C) and necrotic and degenerative lesions of liver parenchyma of lake whitefish fed diets contaminated with U (B & D). (A) A low magnification micrograph of a control lake whitefish liver illustrating the regular cord-like architecture of parenchyma. (B) A low magnification micrograph of a necrotic lesion in the liver of a lake whitefish fed 100 µg/g for 10 days. This liver contained multiple necrotic foci, such as the one depicted here, in which hepatocytes were affected in the manner depicted in (D). Foci containing pyknotic nuclei and cell debris from lysed hepatocytes (between the arrows) were also frequent. (C) Liver parenchyma of a control lake whitefish demonstrating the arrangement of hepatocytes in cords (between the arrowheads), generally two-cells thick, separated by liver sinusoids with circulating erythrocytes (small arrow). Hepatocytes contain spherical and prominent nuclei and have well defined cellular membranes. The cytoplasm of hepatocytes is typically replete with lipid and glycogen deposits, seen as unstained regions (large arrow). (D) A high magnification micrograph of the necrotic region of liver seen in (B). Note the pyknotic appearance of hepatocyte nuclei (arrowheads), seen as a notable darkening and reduced size, the irregular (i.e. non-spherical) nuclear morphologies, and the presence of lysed cells (arrows). Haematoxylin and eosin stain.
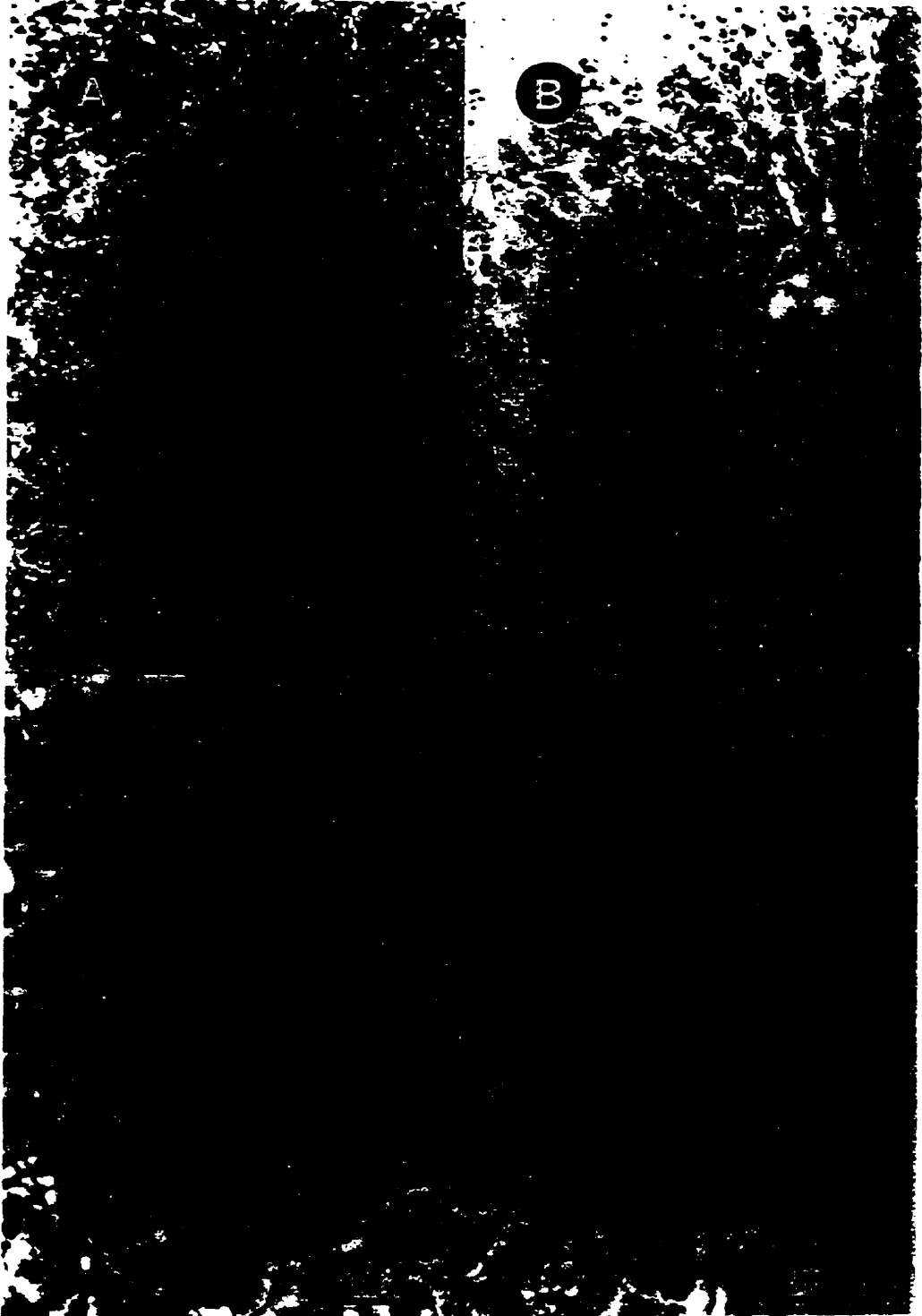
Figure 3.4. The livers of lake whitefish fed uncontaminated diets and a lesion of abnormal architecture and a 'clear focus' in the liver of lake whitefish fed U-contaminated diets. (A) A control lake whitefish liver tissue section. (B) The liver of a lake whitefish fed 10 000 µg U/g for 10 days in which the parenchyma exhibits an atypical architecture. Note the disruption of hepatocyte cords and general randomized arrangement of parenchyma with a tendency towards the occurrence of large masses of hepatocytes (between the arrowheads). Some areas contain enlarged sinusoids (between the arrows), whereas others, most notably the central area, are composed largely of masses of hepatocytes. (C) A region of a control lake whitefish liver parenchyma in which cells are replete with glycogen and lipids (arrowheads). Note that architecture is maintained and hepatocytes contain normal, spherical nuclei (arrows). (D) A 'clear lesion' in the liver of a lake whitefish fed 10000 µg U/g for 10 days. Hepatocytes contain large cytoplasmic vacuoles (clear regions in hepatocyte cytoplasm), likely lipid and/or glycogen, and emarginated and shrunken nuclei (arrow). The swelling of some hepatocytes and the loss of parenchymal architecture has obscured liver sinusoids. Haematoxylin and eosin stain.
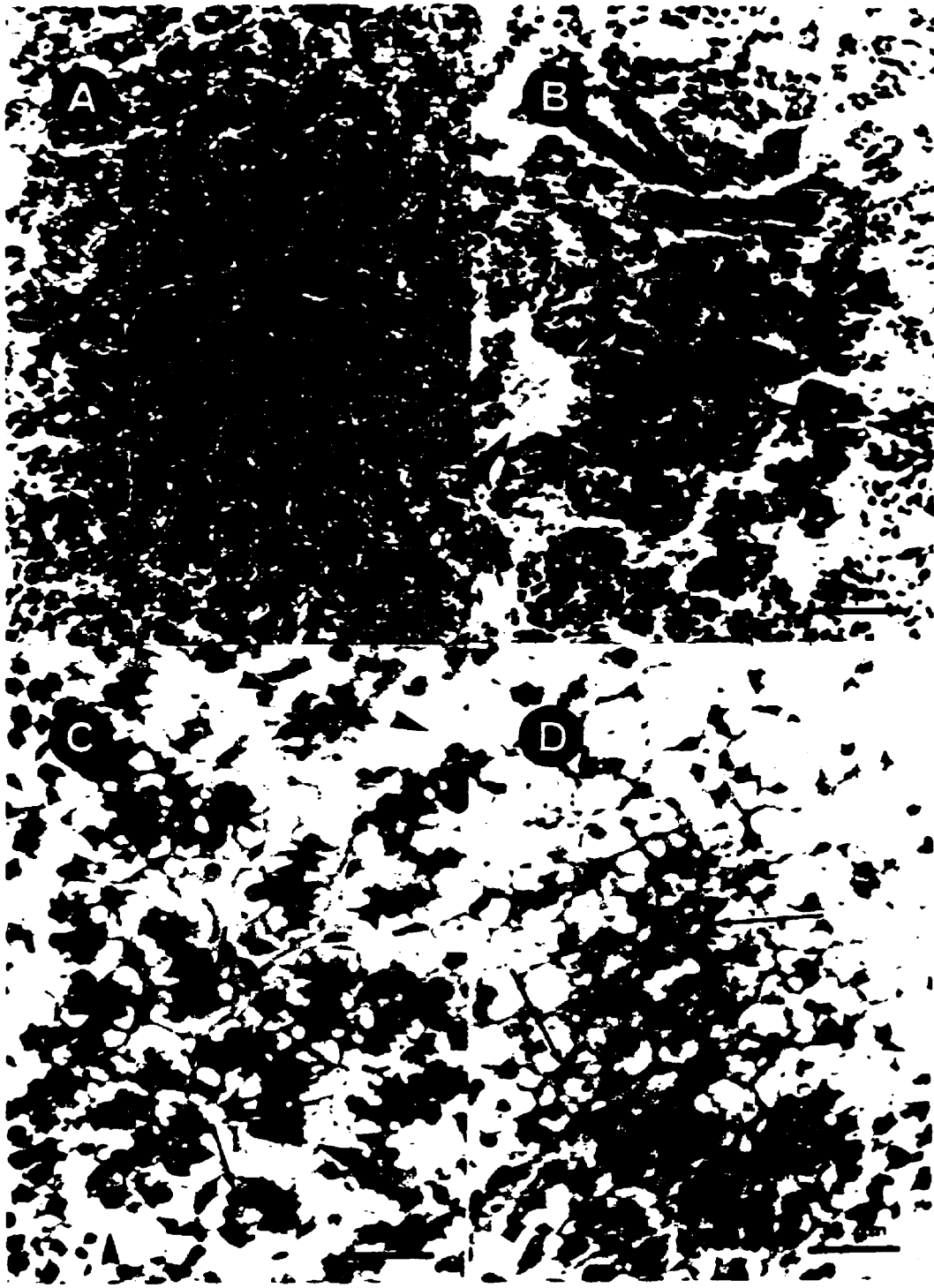
Figure 3.5. The histological appearance of a bile ductule in the liver of a lake whitefish fed an uncontaminated diet and alterations of the bile ductule epithelium in the liver of lake whitefish fed diets contaminated with uranium. (A) A bile ductule and an associated arteriole in the liver of a control lake whitefish. Note the diameter of the bile ductule lumen and the homogenous appearance of the nuclei in the epithelium (arrowhead). (B) Bile ductules and an arteriole in the liver of a lake whitefish fed 100 μg U/g for 30 days. The lumina of the two bile ductules are notably dilated and the epithelium is reduced in height (between the arrowheads). (C) Bile ductules in the liver of a lake whitefish fed 100 μg U/g for 30 days. A number of nuclei of the bile ductule epithelium are enlarged (arrowheads) and contain large, clear vacuoles (arrows). (D) A necrotic bile ductule in the liver of a lake whitefish fed 100 μg U/g for 30 days. Note the pyknotic epithelial cell nuclei (arrowheads) that are desquamating into the lumen, the subsequent partial occlusion of the ductular lumen, and proliferation of surrounding connective tissues (arrows). Haematoxylin and eosin stain. Bile ductule (BD), arteriole (A), lumen (L).

Figure 3.6. Necrotic and other degenerative lesions in the livers of lake whitefish fed an uncontaminated diet and a diet contaminated with 100, 1000, and 10 000 µg U/g for (A) 10, (B) 30, and (C) 100 days. Data are expressed as the number of fish within each treatment group rated in 1 of 4 lesion categories: negligible, minor, moderate-severe, and massive.
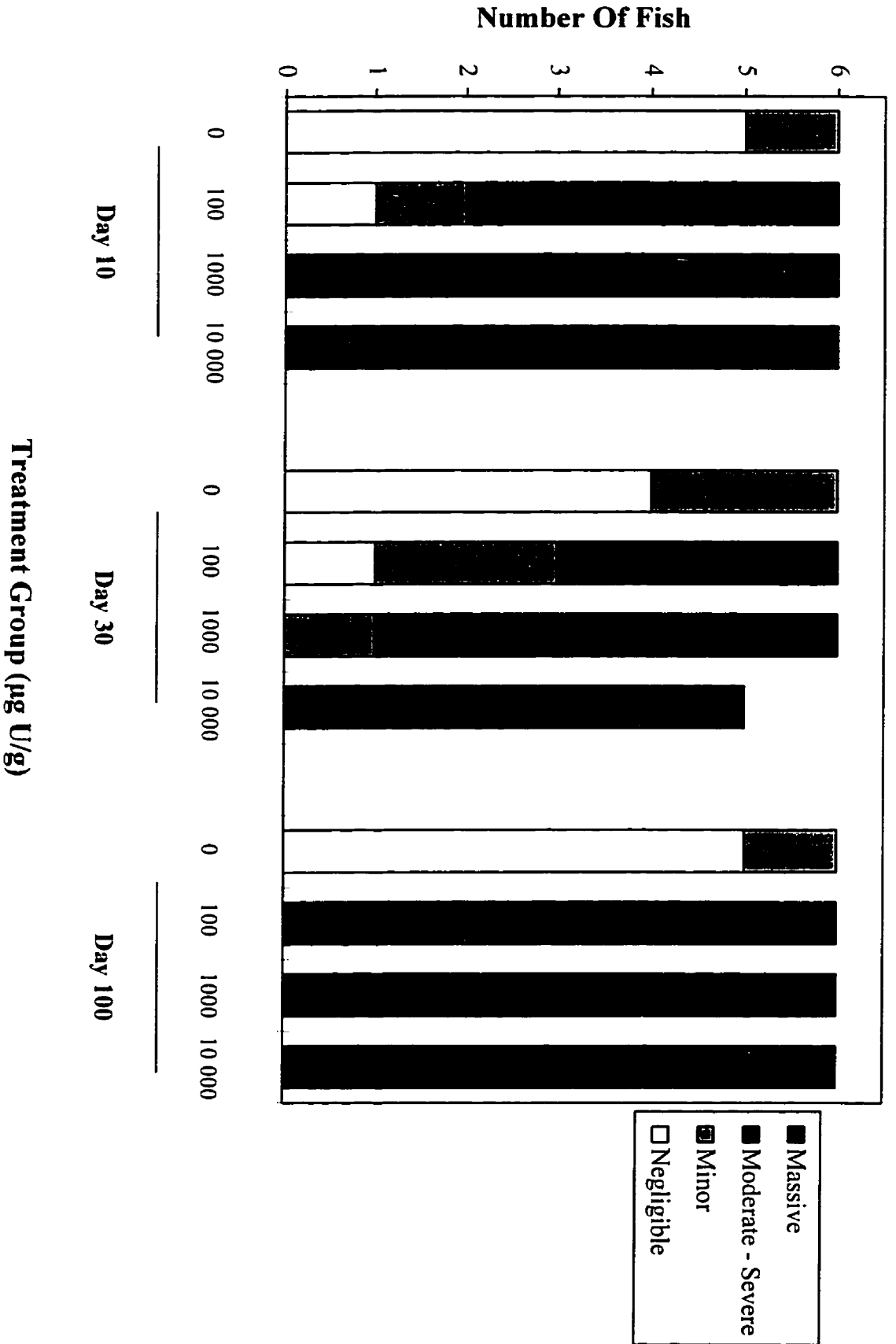
Figure 3.7. Basic structure of the freshwater teleost nephron. (G) glomerulus, (N) neck segment, (P1) first segment of the proximal tubule, (P2) second segment of the proximal tubule, (DT) distal tubule, (CD) collecting duct, and (MD) mesonephric duct. Modified from Hickman and Trump (1969).
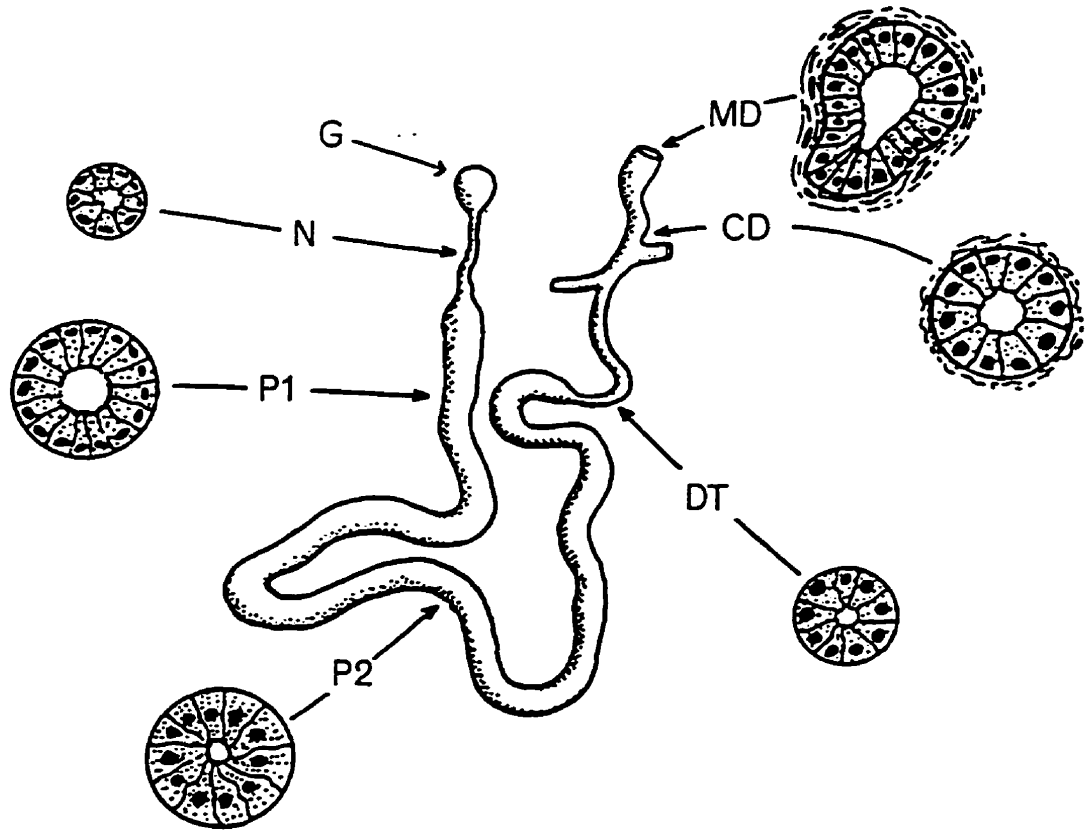
Figure 3.8. The basic histology of the posterior kidney of a lake whitefish fed an uncontaminated diet and renal tubule lesions and pigmented macrophage proliferation in sections of the posterior kidneys of lake whitefish fed diets contaminated with U. (A) The posterior kidney of a control lake whitefish showing various segments of the nephron and interstitial tissues: glomerulus, haematopoietic tissue, first and second segments of proximal tubule, distal tubule, and a blood sinus (arrow). (B) A higher magnification of necrotic tubules (precise segment unidentifiable), exhibiting pyknotic nuclei and desquamating epithelium, in a lake whitefish fed 10 000 µg U/g for 100 days. Also seen are areas of depleted haematopoietic tissues (inside the arrowheads) and peritubular inflammation (arrows). (C) A focus of dilated tubules (between the arrowheads) containing large amounts of eosinophilic debris in a lake whitefish fed 1000 µg U/g for 100 days. (D) The posterior kidney of a lake whitefish fed 100 µg U/g for 100 days exhibiting degenerating tubules and focal proliferation of pigmented macrophages. Note the non-encapsulated aggregation of PM in the centre of the field (between the arrowheads). Haematoxylin and eosin stain. Glomerulus (G), haematopoietic tissue (HT), first (P1) and second (P2) segments of proximal tubule, (DT) distal tubule, necrotic tubule (NT), pigmented macrophage (PM).

Figure 3.9. Micrographs of renal tubules and mesonephric ducts of lake whitefish. (A) Hyalinization of the first segment of proximal tubules in a lake whitefish fed 10 000 μg U/g for 30 days. Arrows denote large cytoplasmic hyaline deposits, although smaller ones can be seen throughout the three P1 in the field. The P1 brush borders (between the arrowheads) are reduced in height. (B) Nuclear alterations of the distal tubules of lake whitefish fed 10 000 μg U/g for 10 days. Note nuclear vacuolation (arrows) and displacement of nuclei toward the luminal membrane (arrowhead). (C) A mesonephric duct in a control lake whitefish. Note the absence of debris in the lumen. (D) A degenerating mesonephric duct in a lake whitefish fed 10 000 μg U/g food for 100 days. The lumen contains pyknotic nuclei and cell debris (between the arrows). Haematoxylin and eosin stain. First segment of the proximal tubule (P1), mesonephric duct (MD), lumen (L).

Figure 3.10. Glomeruli in the posterior kidneys of lake whitefish fed an uncontaminated

diet and glomerular lesions in lake whitefish fed U-contaminated diets. (A) A glomerulus

in a control lake whitefish. Shown are a capillary (between the arrowheads), erythrocytes

(small arrow), and Bowman's capsule (large arrow). Ultrafiltrate passes into Bowman's

space, which is continuous with the lumens of proximal tubules. (B) A glomerular lesion

in a lake whitefish fed 10 000 μg U/g for 100 days. Note the pyknotic nuclei (arrows) and

rounded cells (arrowheads), which appear to be erythrocytes, containing nuclei reduced in

size. (C) A thrombus in an enlarged glomerulus of a lake whitefish fed 10 000 μg U/g for

30 days. (D) Glomerular hyalinization (arrows) in a lake whitefish fed 10 000 μg U/g for

30 days. Haematoxylin and eosin stain. Bowman's space (BS), glomerulus (G), thrombus

(T).
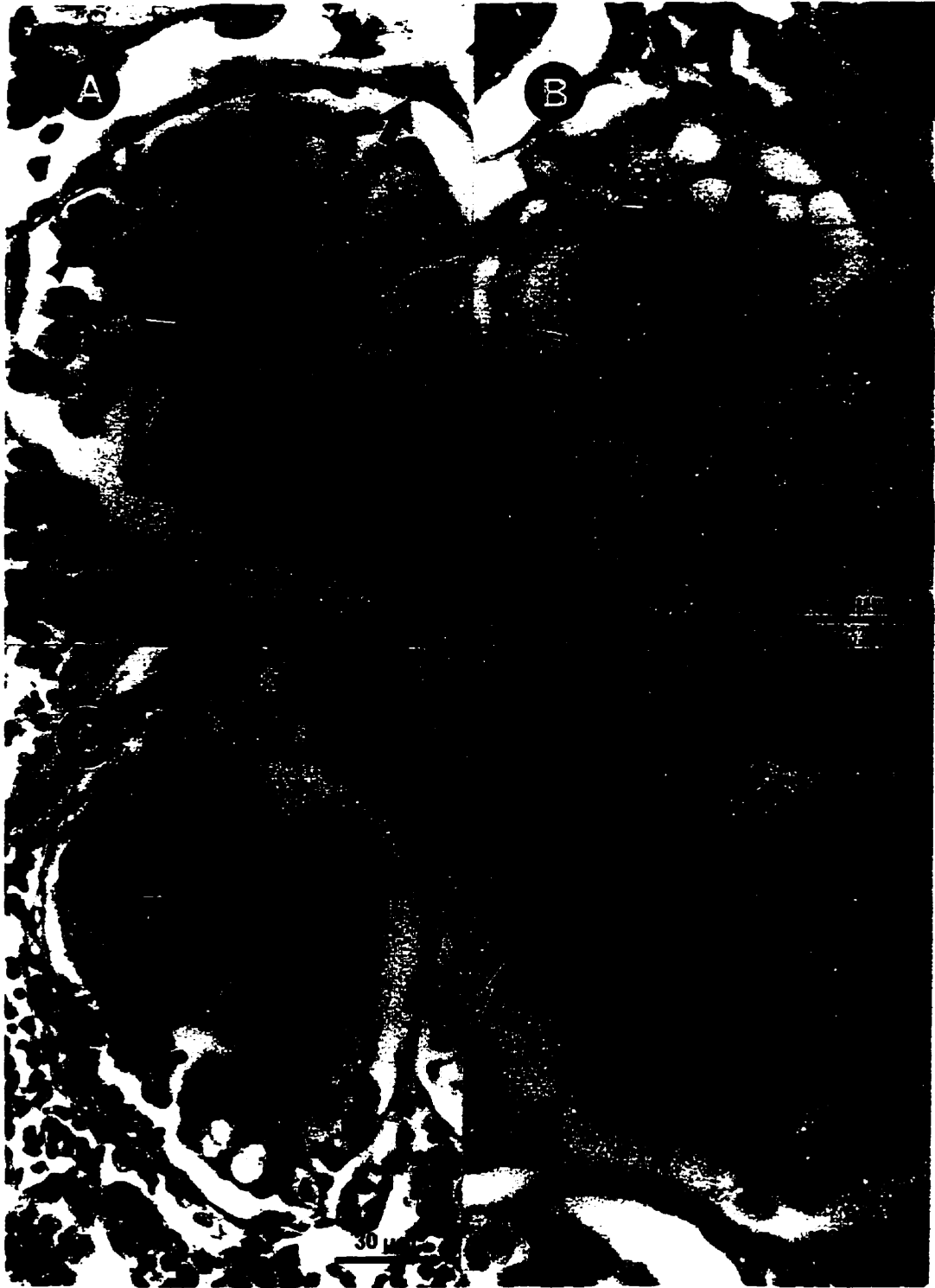
Figure 3.11. Frequency distribution of damaged proximal tubules (P2) in lake whitefish fed an uncontaminated diet and diets contaminated with U for 10, 30, and 100 days. The histological appearance of 15 P2's per fish were categorized according to the degree of alteration observed: (1) normal, (2) minor, (3) moderate, and (4) major. The data obtained for each fish were then pooled within each treatment group, and expressed as a percentage (from a total of 90 tubules) of tubules per treatment group assigned to each of the above categories.
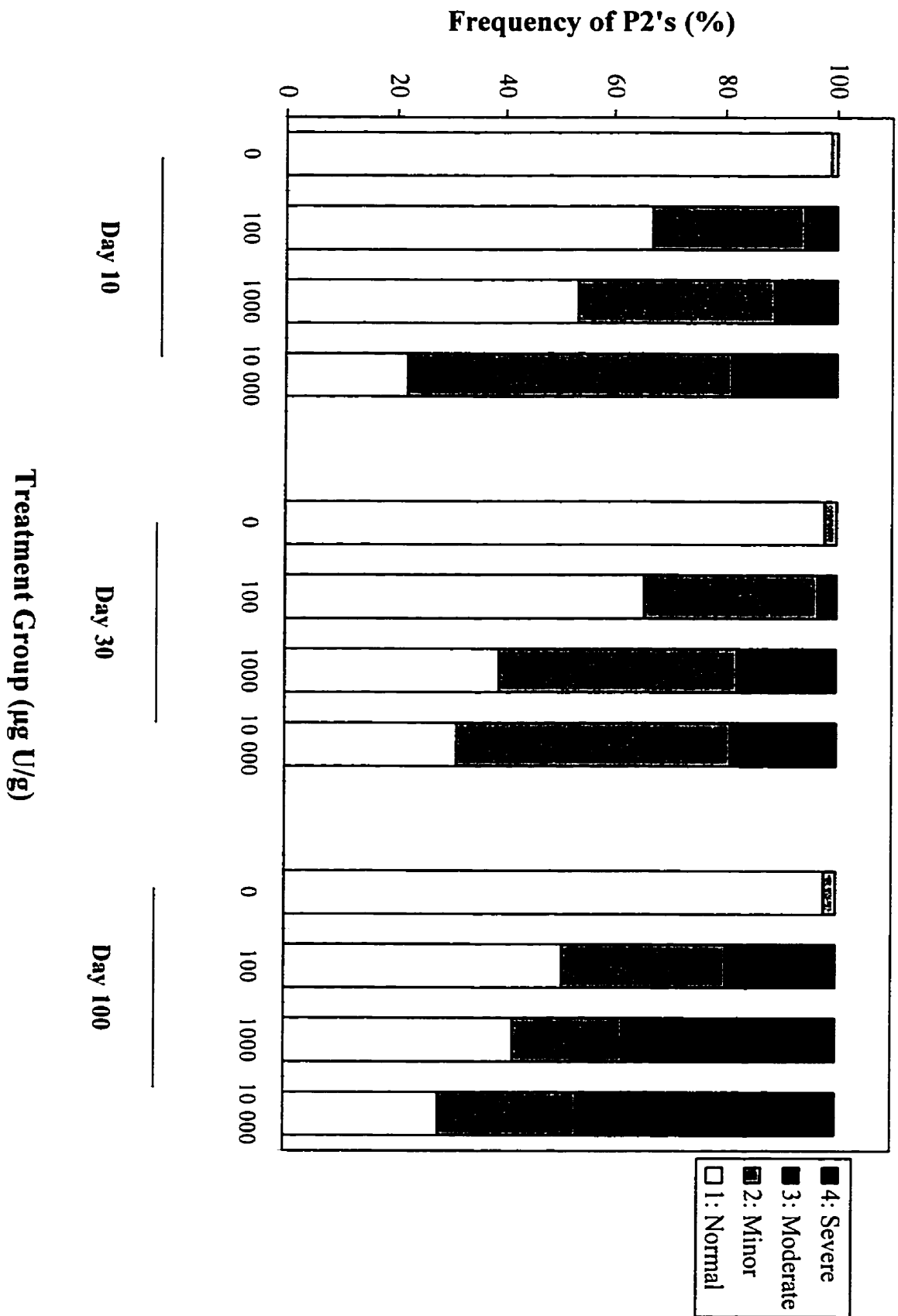
Figure 3.12. Frequency distribution of damaged proximal tubules (P1) in lake whitefish fed an uncontaminated diet and diets contaminated with U for 10, 30, and 100 days. The histological appearance of 15 P1's per fish were categorized according to the degree of alteration observed: (1) normal, (2) minor, (3) moderate, and (4) major. The data obtained for each fish were then pooled within each treatment group, and expressed as a percentage (from a total of 90 tubules) of tubules per treatment group assigned to each of the above categories.
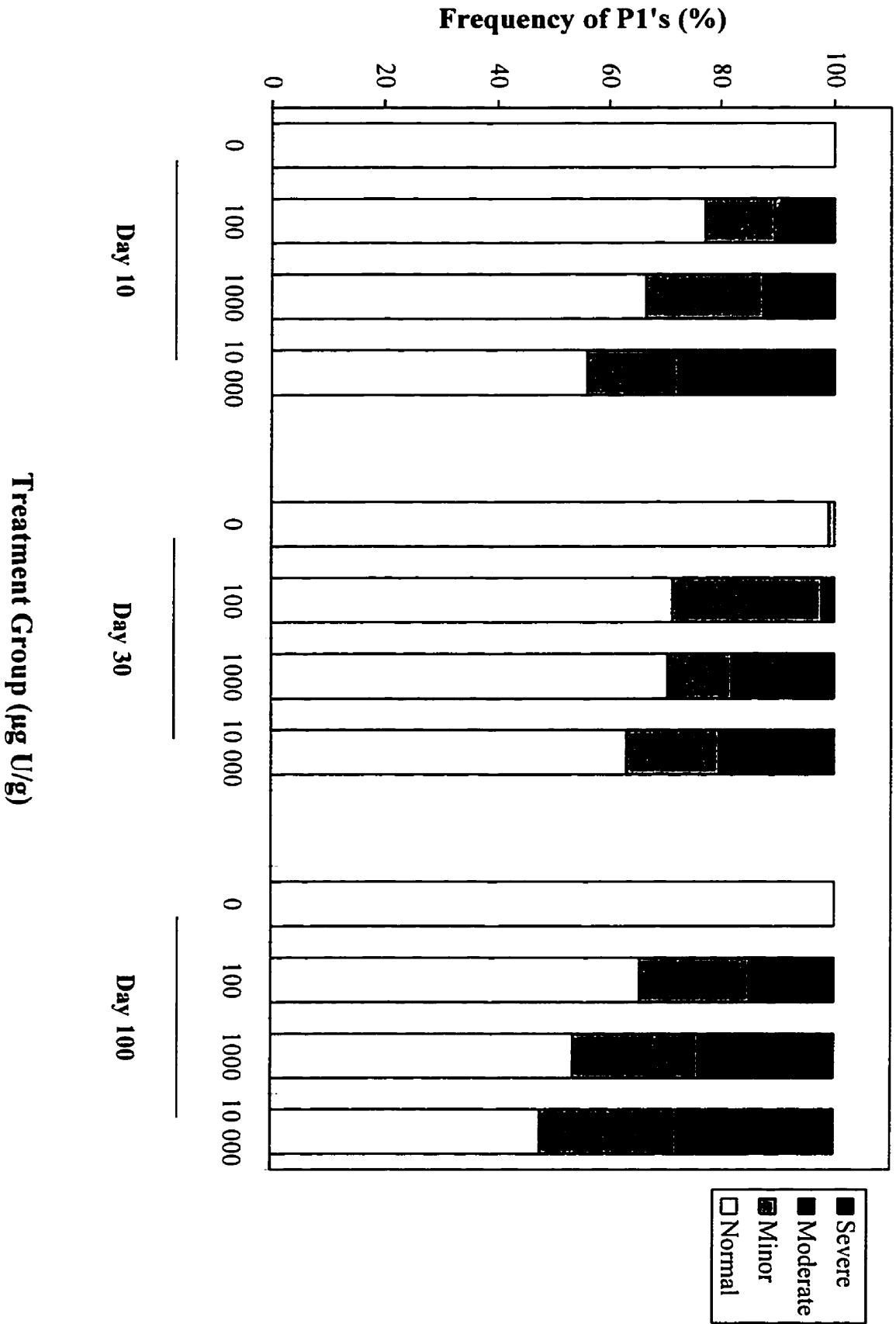
Chapter Four

General Discussion

The following discussion is aimed at providing more detailed interpretations of the results of this study and an integration of results presented in chapters two and three. The implications and environmental relevance of this research are also addressed. In addition, the potential radiological hazards associated with concentrations of U in the tissues of lake whitefish are evaluated. Although it is widely recognized that U is more chemotoxic than radiotoxic (Wrenn et al. 1985), the issue of radiation was evaluated for three reasons. Firstly, it was necessary to confirm that the effects observed in lake whitefish were not a consequence of radiation. Secondly, feral fish are exposed to natural U ore which has a higher specific activity than the uranyl acetate used in this study. Thirdly, U decay produces highly radioactive daughter progeny to which feral fish are exposed. Therefore, an examination of this nature is relevant in the context of exposures typical in the natural environment.

I. Discussion of U Exposure and Accumulation

A. Bioavailability: Uranium Speciation

One objective of this study was to address the bioavailability of ingested U. Because fish from all exposure groups accumulated U in at least one tissue by the end of the exposure period, the results indicate that U is absorbed from the gastrointestinal tracts of lake whitefish and is distributed in various mineralized and soft tissues. Although the species of U contained in the food was not analyzed directly, the U contained in the

source chemical exists in the hexavalent oxidation state in the form of the uranyl ion, UO_2^{2+}, the most bioavailable form of U (Sullivan et al. 1986, Tannenbaum 1951). The uranyl ion is the most soluble species of U and forms a yellow coloration in solution, whereas the other two soluble U species form blood-red and green solutions (Considine 1989). As the U solution applied to fish food was yellow, it is reasonable to assume that the U added to the food was in the hexavalent oxidation state. This same species is believed to predominate in biological tissues (Tracy et al. 1992).

B. U Accumulation: Mechanisms of Absorption

The present study was not designed to evaluate the mechanism(s) by which U is absorbed across the gastrointestinal tract of fish, however, there may be similarities to mechanisms observed in mammalian systems. Generally, very little ingested U is taken up across the gastrointestinal tracts of mammals. There is a wide range of suggested transfer coefficients in mammals; from 0.06% in rats and rabbits (Tracy et al. 1992) to as high as 30% in humans (Hursh et al. 1969). A critical review of existing human and non-human animal data generated a suggested value of 1-2% (Wrenn et al. 1985). In agreement, the ICRP has recently revised the gastrointestinal absorption factor to 2% (Leggett 1994).

U appears to penetrate the gut barrier in rats gavaged with ^{233}U, by binding to iron-binding proteins, such as transferrin (Sullivan and Ruemmler 1988). Iron-deficient rats accumulated three to four times more U in liver, kidney, and bone, than iron-replete rats (Sullivan and Ruemmler 1988), suggesting that dietary U may be absorbed by an iron

transport system. Furthermore, ^{233}U, in the hexavalent oxidation state, has a strong affinity for transferrin. Approximately 40% of plasma U is complexed to this fraction (Stevens et al. 1980) and U-transferrin complexes in blood are the most stable of all the circulating ligands (Ribera et al. 1996). The uptake of other metals, including Pb (Hamilton 1978) and Pu (Sullivan and Ruemmler 1988), via iron-binding pathways has been confirmed. Lead is believed to behave similarly to U in biota (Leggett 1994) and U has been used as a simulator of Pu in surface waters (Boniforti 1987).

U's avidity for iron binding proteins has been suggested as a potential mechanism of U accumulation in mammals that have inhaled nuclear fuel particles. Rat pulmonary macrophages accumulate UO_2 particles in lysosomes by a process of phagocytosis (Muller et al. 1989). However, the particles may be solubilized and leave the lysosome where they bind to cytosolic ferritin. Some U also leaves the cells, and is transported from the extracellular fluid to the blood, likely by binding to transferrin.

The ICRP has recently replaced its biokinetic model for U, which was similar to those applied to other actinide elements, including Pu. The current model is a modification of those applied to the alkaline earth elements, barium, strontium, radium, and lead (Leggett 1994). All of these latter elements are bone-seekers and are believed to behave as metabolic analogues of Ca. Similarly, because U displaces Ca^{2+} in the mineral matrices of mammalian (Neuman 1953, Rowland and Farnham 1969) and teleost bone (Kovalsky et al. 1967), it is reasonable to speculate that U may also cross the gastrointestinal tracts of organisms using Ca transport pathways.

Several mechanisms for the gastrointestinal transfer of nuclear fuel compounds, including UO_2, have been demonstrated in mammals (Lang et al. 1995). Large particles with a low solubility may be taken up by the lymphoid elements present on intestinal mucosa by phagocytosis. Another proposed mechanism for the uptake of large particles is persorption. This process occurs as the epithelial cells of the villi are sloughed, creating a temporary gap in the membrane through which membrane-impermeable particles may penetrate. Lastly, particulate radionuclides may enter the gastrointestinal epithelium as a result of membrane damage. All of these mechanisms may be operating in the present study, and possibly in the natural environment.

U has been demonstrated to interact with phosphatase enzymes and cathepsins, a family of proteolytic enzymes, both of which are fundamental components of fish gastrointestinal systems (Kuz'mina 1995). The accumulation of U on microorganisms, such as *Citrobacter* sp., is mediated by the activity of a phosphatase enzyme which liberates HPO_4^{2-}, the substrate on which U binds (Yong and Macaskie 1995). The enzyme is inhibited by U at high concentrations, except in the presence of excessive quantities of substrate.

Intraperitoneal injections of U increased the activities of alkaline phosphatase and cathepsin D in carp liver, responses which were recommended for use as biological indicators of U pollution (Lee et al. 1994). Stimulation of acid phosphatase activity may be the mechanism by which U is accumulated intracellularly in the form of a microneedle phosphate crystal within the lysosomes of a number of biota (Chassard-Bouchaud 1982, 1983, Chassard-Bouchaud and Hallegot 1984, Galle 1974, Galle et al. 1992). The

mechanism of accumulation in the lysosomes of marine crabs and mussels (Chassard-Bouchaud and Hallegot 1984) and mammals (Galle et al. 1992), involved the activity of an intralysosomal acid phosphatase. These observations indicate a potential for U to interact with enzymes present in fish intestine, and possibly a mechanism of accumulation in this, and other, tissues. Further investigations on these interactions are required to elucidate mechanisms of intestinal absorption and deposition in other tissues of fish.

C. U Accumulation in Lake Whitefish Tissues

Intestines

Although accumulation of U in lake whitefish intestine was substantial, there is a paucity of data to which these results may be compared. However, concentrations of U in the stomachs of feral fish residing in northern Saskatchewan have been reported. Fish inhabiting Beaverlodge Lake, Saskatchewan, a system contaminated by U mining and milling discharges, consistently contained the highest concentrations of U in the stomach (Swanson 1985). Mean ($\pm$ SE) concentrations in the stomachs of lake whitefish and white sucker were 165.08 $\pm$ 49.28 μg/g (ww) and 85.99 $\pm$ 13.97 μg/g (ww), respectively. Even fish sampled from nearby reference systems contained detectable amounts of this contaminant in the stomach; concentrations ranged from 1.90 $\pm$ 0.44 μg/g (ww) to a maximum of 18.04 $\pm$ 6.27 μg/g (ww), the highest of all tissues analyzed. Presumably, the

presence of U in the tissues of reference fish is a reflection of the location of reference lakes in an uraniferous region.

It is not clear, however, whether stomach contents were included in the analysis of stomach samples in the latter study (Swanson 1985). If they were, these values may overestimate the concentration of U that is assimilated into stomach tissues, because stomach contents generally contain higher concentrations of U than the soft tissues of fish (e.g. Waite et al. 1988). If stomach contents were removed, concentrations reported by Swanson (1985) are high, relative to the results obtained in the present study. For example, lake whitefish fed a diet containing 1000 μg U/g food for 100 days accumulated 14.0 ± 2.3 μg U/g in intestine. This concentration is very similar to the mean concentration, 18.04 ± 6.27 μg/g (ww), observed in the stomachs of lake whitefish taken from one of the reference systems included in the survey of the Beaverlodge Lake area (Swanson 1985). Concentrations of U in water (3.25 ● 1.64 μg/L) and sediments (6.60 ± 1.72 μg/g ww) (Swanson 1985), although above background for Canadian systems, were considerably lower than dietary concentrations used in the present study.

Conversely, lake whitefish sampled in Langley Bay, Lake Athabasca, Saskatchewan, contaminated by tailings deposited by the Gunnar Uranium Mine, contained the highest concentration of U in bone, followed by kidney (Waite et al. 1988). Although detected, the concentration of U in gut, 0.1 μg U/g, was six times lower than that observed in kidney and only twice as high as that found in male gonads and muscle (Waite et al. 1988). This relative tissue distribution conflicts with those observed in fish

fed moderate and high concentrations of U in the present study, but is more in agreement with results obtained from the low treatment group.

Because U could be detected only in the scales of fish from the lowest treatment group, these data indicate that at low exposure concentrations, such as those encountered in Langley Bay (Waite et al. 1988), U may accumulate largely in mineralized tissues such as bone and scales and disproportionately less in the intestines. It should be noted, however, that the entire gut was analyzed in Langley Bay lake whitefish (Waite et al. 1988). It may be inferred that the low concentrations reported by Waite et al. (1988) are a consequence of diluting the sample with the upper portions of the GI tract which may contain lower concentrations of metals than the posterior intestines of fish. For example, feral brown trout inhabiting the metal-contaminated Clark Fork River, Montana contained higher concentrations of Cd and Cu in the large intestine than either the stomach or the pyloric caeca (Farag et al. 1995).

Furthermore, because the detection limits in the present study were tissue-dependent, where these limits were not exceeded it becomes difficult to comment upon relative differences in tissue accumulation. Because the analytical detection limits are in fact higher for intestine than for scales, due to differences in the weights of tissues available for analysis, it can not be empirically stated that accumulation is in fact significantly greater in the scales of fish fed low concentrations of U, relative to intestine.

Bone and Scales

U displayed a particular affinity for lake whitefish bone and scales. Observations on the accumulation of U in the skeleton of mammals may translate to enhanced accumulation of U in the bones of young and actively growing fish. U does not accumulate homogeneously in all bones of mammals (Hamilton 1972, Singh et al. 1987b, Welford and Baird 1967). Rather, it is preferentially deposited in bones with a high surface area, high vascularity, and those with high metabolic activity. Thus, young fish may accumulate higher U concentrations than older fish, as is observed in children relative to adults (Lianqing and Guiyun 1990). Furthermore, fish may be at greater risk of accumulating U in bone, because they experience indeterminate growth. In addition, the half-time for retention of U in the long component of skeletal tissues of mammals is extremely high; estimates reach as high as 5000 days in humans (Tracy et al. 1992). This indicates a potential for very long-term retention in fish bone.

The accumulation of U, and other contaminants, in bone has largely been heralded as fortuitous because it effectively removes U from the circulation and prevents interaction with vulnerable soft tissues. Thus, this affinity for mineralized tissues has been deemed a detoxification mechanism. This interpretation is questionable, however, as it has become apparent that U may be remobilized from mammalian bone in the short-term, by cation exchange at the bone surface, and in the long-term, through bone resorption (Leggett 1994). Hence, the long-accepted paradigm that the accumulation of U in bone functions as an inert sink is dubious. It is analogous to the view that aquatic sediments serve a similar 'sink' function with regard to contaminant accumulation in the environment.

Alternatively, accumulation of U in bone, particularly given its long biological half-time, may be viewed as a chronic hazard to biota, because a slow release of U from this pool will serve as a long-term source of U to the soft tissues, even if exposure is discontinued. Furthermore, while the kidney is the primary target tissue for U's chemotoxicity, the bone is considered the critical site of U's chronic radiotoxic action in mammals (Lloyd et al. 1996, Mays et al. 1985, Stevens et al. 1980). It is also conceivable that the skeletal tissues of fish could be damaged by U's chemotoxic action as has been observed in mammals (Guglielmotti et al. 1984).

Arguments similar to those cautioning the view that the deposition of U in bone is protective, have emerged regarding the deposition of metals in fish scales. A portion of the Zn deposited in the scales of mummichog (*Fundulus heteroclitus*) exposed to elevated levels in water was removed following transfer of fish to clean water, indicating that at least for Zn, remobilization from this tissue is possible (Sauer and Watabe 1989). The precise mechanism by which this occurs is not established but appears to occur by scale resorption. As argued for bone, U deposited in lake whitefish scales may also be released as a consequence of scale resorption. As this physiological process is critical during periods of gonadal maturation and reproduction and during periods of starvation (Yamada 1956), if U does accumulate in the mineral component of fish scales, U could be released into the circulation during these critical times in the life history cycle. Furthermore, the accumulation of U in fish scales could cause structural damage to this tissue, similar to that caused by dietary (Yoshitomi et al. 1998) and aqueous (Rishi and Jain 1998) cadmium in carp (*Cyprinus carpio*).

Liver

In the mammalian biokinetic model of U, it is accepted that only a small fraction of ingested U accumulates in liver (Leggett 1994). The results of the present study, and those of Swanson (1985), suggest that fish may accumulate proportionately more U in liver than mammals. This may be attributable to differences between the relative role of the teleost and mammalian biliary systems in eliminating U. In general, a number of other metals are excreted in the bile and, in turn, accumulate in the hepatic tissues of fish (Handy 1996). Furthermore, when metals are introduced in the diet, the biliary route of excretion may be more important than when fish are exposed to waterborne metals (Handy 1996). The occurrence of liver tissue damage in lake whitefish (chapter three), in particular lesions of bile ductule epithelium, indicates that biliary excretion of U may be significant in fish.

Gonads

The accumulation of U in lake whitefish gonads was considerable in fish from the highest treatment group. However, the temporal accumulation was non-linear, as the highest concentrations were observed on sampling day 30. Furthermore, although not subjectable to statistical evaluations, there appeared to be greater accumulation in the testes than the ovaries of these fish.

Accumulation of U in gonads of feral fish inhabiting environments contaminated by U mining and milling activities, yield conflicting and unclear results. Lake whitefish

and white sucker residing in the U-contaminated Beaverlodge Lake, Saskatchewan contained notable U residues in gonads (Swanson 1985). Of the various tissues analyzed, U concentrations in gonads surpassed only those in muscle. No sex-related disparities in accumulation were apparent. Analysis of U in the same species inhabiting Milliken and Fredette Lakes, the two reference systems, yielded contradictory results. In the first system, U accumulated in lake whitefish gonads at concentrations similar to those observed in skin. In the second system, lake whitefish gonads contained similar concentrations as the livers - the third greatest concentrations observed in the five tissues analyzed. Conversely, white suckers from Milliken Lake contained the lowest concentrations of U in gonads, about equal to concentrations measured in muscle.

A seasonal trend in U accumulation was reported for lake whitefish and white sucker from Beaverlodge Lake (Swanson 1985). Temporal disparities in U concentrations, highest during the spring sampling period relative to the fall, did not coincide with spawning and non-spawning fish, but rather, were likely attributable to seasonal differences in feeding rates, and/or bioavailability of metals. Unfortunately, data were not presented by season, nor were observations on spawning, thus impeding further comparisons with the present data.

. Lake whitefish from Langley Bay, Saskatchewan contained concentrations of U in the order: gut content > bone > kidney > liver = ovaries > gut > male gonads = muscle. These tissues were analyzed, however, as composites of 12 fish and were not subject to statistical evaluation (Waite et al. 1988). Generally, concentrations in all tissues were lower than those reported in lake whitefish inhabiting Beaverlodge Lake. In fact,

concentrations were remarkably similar to those measured in lake whitefish from the two reference lakes included in the evaluation of the Beaverlodge Lake area (Swanson 1985). Concentrations were four times greater in ovaries than in male gonads of fish inhabiting Langley Bay (Waite et al. 1988), however, without any measure of variance these differences are dubious. If this apparent trend is representative of sex-related differences in U accumulation, it is in direct conflict with the results of the present study.

One aspect relevant to accumulation of U in gonads is the potential for fish to maternally transfer U. The incorporation of U into fish ova, an occurrence reported by Kovalsky et al. (1967), may pose a chemical, and possibly radiological, hazard to developing embryos. This transfer is critical due to the inherently high sensitivities of the early life cycle stages of fish to both radiation (Bonham and Welander 1963, Templeton 1980) and the chemotoxicity of metals and metalloids, including Al (Palmer et al. 1989), As (Eisler 1988), Hg (Birge et al. 1979), Pb (Davies et al. 1976), and Zn (Spear 1981). The sensitivities of cold- and warm-water fish species to aqueous U also appear to decrease with age (Appendix four).

It is plausible that U accumulates in developing ova by binding to the phosphate-containing protein, vitellogenin (VTG), which functions as the precursor to yolk proteins (Tyler and Sumpter 1996). Because U has an affinity for phosphates in the abiotic (Abu-Hilal 1994) and biotic (Chassard-Bouchaud 1982, 1983, Chassard-Bouchaud and Hallegot 1984, Galle 1974) environments, it is reasonable to postulate that U may accumulate in fish oocytes by binding to VTG. The maternal transfer of other metals into fish ova has been confirmed. Portions of excessive levels of Cu and Zn in white suckers

inhabiting a contaminated environment appear to have been maternally transferred to eggs in association with yolk precursors, and subsequently mobilized during yolk resorption (Munkittrick and Dixon 1989). It was also demonstrated that Cd binds to VTG and is deposited in the ovaries of fish *in vivo* (Ghosh and Thomas 1995). *In vitro* experiments further demonstrated that Cd is capable of displacing the *in situ* metals calcium and zinc from their VTG binding sites.

D. U Accumulation: The Potential Role of Phosphates

Because U accumulates in highly mineralized tissues of lake whitefish, its distribution may be governed by the phosphate content of these tissues. Some evidence derived from observations on the behaviour of U in abiotic and biotic systems provides credence to this possibility. Uranium has a strong geochemical association with phosphates, a characteristic which explains its high content in phosphate rocks, fertilizers, and high phosphate-containing sediments (Abu-Hilal 1994). Uranium is also a significant component of marine corals, both living and dead, a phenomenon explained by its chemoattraction for carbonates and phosphates (Abu-Hilal 1994). In addition, U has a high affinity for phosphates and carbonates in the hydrological environment when in the hexavalent oxidation state, the principle species of U found in aquatic environments (Poston et al. 1984). It is because of this propensity to complex with these abundant anions that U is extremely mobile in surface waters (Cowart and Burnett 1994, Poston et al. 1984).

Within biota, U exhibits chemical behaviours similar to those observed in the abiotic environment. Its hydrological and geochemical tendency to complex with phosphates is also observed in living organisms, both at the tissue and subcellular levels. At the higher level of biological organization, U manifests its attraction for phosphates as a tendency to accumulate in high concentrations in tissues with a high phosphate content. This proclivity materializes as high U concentrations in the bone of mammals (Leggett 1994) and fish (Bernstein and Swanson 1989, Koval'skiy and Vorotnitskaya 1965, Swanson 1982, 1983, 1985, Waite et al. 1988), in the carapace and shells of crustaceans and molluscs (Chassard-Bouchaud 1983, Koval'skiy and Vorotnitskaya 1965), and in the calcareous walls of algae (Koval'skiy and Vorotnitskaya 1965).

Similarly, at the subcellular level, U associates with phosphates, generally existing as a mineral precipitate. In mammals, U accumulates in the lysosomes of the kidney, where it precipitates in the form of U-phosphate microneedle crystals (Galle 1974). Likewise, within the tissues of laboratory-exposed marine crabs (*Carcinus maenus*), U was localized in intra- and extracellular spherocrystals consisting of calcium phosphate granules, as well as in lysosomes in the form of uranium phosphate microneedles (Chassard-Bouchaud 1983). Similarly, U was consistently found in association with phosphorous in the lysosomes of marine crabs and mussels inhabiting polluted French waters (Chassard-Bouchaud and Hallegot 1984) and when exposed in the lab (Chassard-Bouchaud 1982). Freshwater hydra (*Hydra viridissima*) accumulate U as a phosphate crystal in discharged nematocysts (Hyne et al. 1992a). The collagenous capsule degenerates following nematocyst discharge and associates with the U.

Furthermore, U accumulates in the nuclei as a phosphate precipitate in mussels, shore crabs, and freshwater crayfish (Chassard-Bouchaud 1982).

In addition, uranium's affinity for phosphates is exploited in the treatment of U poisoning. One injection of the phosphate-containing chelating agent ethane-1-hidroxy-1,1-biphosphonate increased the survival and prevented histopathologies in the kidneys of rats (no deaths occurred) exposed to acutely lethal doses of U by injection (Ubios et al. 1994). This pharmaceutical is widely used in the treatment of osteopenic diseases in humans. All of these observations suggest that U deposition in tissues and within tissues may be governed by the presence, and perhaps abundance, of phosphates.

The relative tissue distribution of U in lake whitefish, most effectively demonstrated in the high treatment group, revealed an interesting trend. In accordance with the known tendency for U to associate with phosphates geochemically and within living systems, the distribution of U in lake whitefish tissues parallels the relative distribution of phosphates in freshwater fish. The compartmentalization of radionuclides, consisting mainly of ^{32}P, in the tissues of whitefish (species unreported) inhabiting the Columbia River, contaminated by the Hanford reactors, was similar to the distribution of U in lake whitefish from the present study (Davis and Foster 1972). It was concluded that ^{32}P could be deemed representative of the distribution of phosphates among tissues (Davis and Foster 1972).

Of particular interest are the bone and scales, because they contained both the highest concentrations of ^{32}P in Columbia River whitefish (Davis and Foster 1972) and high concentrations of U in laboratory lake whitefish. Furthermore, the muscle, which did

not accumulate significant concentrations of U in lake whitefish, also contained the lowest concentration of ^{32}P in whitefish from the Columbia River (Davis and Foster 1972).

From examination of the current literature on U and considering the results of this study, it appears that U associates with phosphates *in vivo*, a behaviour which may in turn, determine, at least in part, its relative distribution in freshwater fish tissues. Further research on the subcellular and molecular sites and the mechanisms of U accumulation in the tissues of freshwater fish is required in order to ascertain the role of phosphates in governing these processes.

II. Discussion of Effects Examined

A. Morphometrics

A lack of toxic manifestations at the whole organism level (i.e. fish morphometrics), as was observed in this study, is not unusual (e.g. Lowe-Jinde and Niimi 1984) and attests to arguments for inclusion of more sensitive indicators of contaminant toxicity in evaluations of fish health. These results are of significance to monitoring of feral fish in environments impacted by U mining and milling. Because the results of this study demonstrated toxicity by evaluations at the tissue and biochemical levels but not at the whole organism level, monitoring programs restricted to the latter type of health

assessments may fail to identify the occurrence of sub-lethal toxicity in feral fish exposed to U.

B. Haematology

U exposure caused few and only transient disturbances in the haematological profiles of lake whitefish. The increase in serum osmolality in lake whitefish fed 10 000 µg U/g for 30 days could have been caused by numerous mechanisms. In part, concomitant elevations in serum Cl⁻ and lipid peroxides would contribute to this condition. Increases in a number of other substances, such as calcium, glucose, lipids, proteins, or enzymes, could heighten osmolality, all of which have been elevated in fish by contaminant exposure (Mayer et al. 1992). Mammals exposed to U may respond by elevations in plasma glucose and protein or increases or decreases in the enzymes GOT and GPT (Bentley et al. 1985, Ortega et al. 1989).

A second possibility is that the rise in lake whitefish serum osmolality is a manifestation of a decreased ability to excrete nitrogenous wastes. Chronic renal failure in mammals results in a decreased nitrogenous waste output arising from a reduction in GFR, and is typical in mammals exposed to U. The clinical signs of reduced GFR, increases in BUN and/or serum creatinine, have been reported in mammals exposed to U (Bentley et al. 1985, Domingo et al. 1987, Stevens et al. 1980, Stroo and Hook 1977). However, because the principle function of the freshwater teleost kidney is the excretion of large volumes of water and not excretion of nitrogenous wastes (Trump et al. 1975), renal damage in teleosts might be expected to have little effect upon the excretion of

nitrogenous wastes. Nonetheless, effects observed in mammals exposed to U have been produced in teleosts exposed to other metals. For example, Hg and Cu, established nephrotoxins in mammals and fish, increased BUN in teleosts (Lockhart et al. 1972, Singh and Reddy 1990).

Increases in serum Cl^- also observed on day 30 could have developed by numerous mechanisms. Chloride maintains cellular integrity and acid-base balance in the blood and an increase, mediated by stimulation of the Cl^-/HCO_3^- exchanger in gill chloride cells, may occur when concentrations of the bicarbonate ion drop, thus creating a metabolic acidosis (Goss et al. 1995). Metabolic acidosis could have arisen in lake whitefish if a significant fraction of the bicarbonate content of the blood were sequestered by U, effectively decreasing the portion available for normal physiological processes.

The transient nature of both of these haematological lesions is not unusual. Although acute exposure to contaminants, especially via the water, frequently disrupts the concentrations of serum electrolytes, chronic exposures of fish to contaminants may not. Furthermore, where the exposure period is relatively prolonged, initial alterations often disappear even when exposure is continued (McKim et al. 1970). Disruptions in ionoregulatory processes, particularly in intestine and kidney, by contaminants may not alter serum electrolyte concentrations in fish at all. Teleosts have a remarkable capacity to compensate for reduced dietary uptake and excessive urinary losses of nutrients, mainly achieved by enhanced transfer across the gill epithelium. For instance, branchial uptake of Ca^{2+} can completely compensate for an absence of Ca^{2+} in the diet of freshwater fish (Ichii and Mugiya 1983).

Although group mean haematocrits of treated fish did not differ from controls, some individual fish consuming U were notably anaemic. Many contaminants possess some ability to disrupt red cells in fish, the mechanisms of which are multiple (Nikinmaa 1992). A reduction in haematocrit may arise as a result of erythrocyte haemolysis (haemolytic anaemia) caused by increased membrane fragility and subsequent cell swelling. Membrane fragility may be caused by enhanced oxidative stress, which compromises the structural integrity of membranes (Steffens 1989). Other mechanisms include effects at the sites of erythropoiesis, the kidney and spleen, disruptions in iron absorption or metabolism (hypoplastic anaemia), and haemorrhaging (haemorrhagic anaemia) (Roberts 1978). Whatever the proximate cause, all of these pathways may cause a reduction in erythrocyte volume which is evident by measures of packed cell volume, or haematocrit.

The method used here, haematocrit, is incapable of discriminating between the various forms anaemia, and thus does not provide immediate information regarding the mechanism of this effect observed in individuals. Therefore, it is not possible to comment on the direct sites of U toxicity here (i.e. erythrocytes, vascular system, haematopoietic tissues) and all of the aforementioned causes of anaemia could be operating in lake whitefish exposed to U. However, haemorrhaging was common in the posterior kidneys of fish and could have contributed to the anaemic states observed in individuals. Conversely, the presence of increased LPO in serum implicates lipid peroxidation of erythrocyte membranes as a potential mechanism. A third possible explanation for reductions in lake whitefish haematocrits is impairment of iron uptake in the intestine. U

is accumulated from the diet via the iron transport pathway in mammals (Sullivan and Ruemmler 1988). This behaviour could reduce iron accumulation via competition for binding sites on iron binding proteins, such as transferrin.

More prolonged exposure to U may result in consistent reductions in the haematocrits of fish, as has been reported for feral fish populations. Packed cell volumes and red blood cell counts were lower in lake whitefish and white suckers inhabiting Beaverlodge Lake, relative to fish from two reference systems (Bernstein and Swanson 1989). Conversely, the opposite trend was found for lake trout. The presence of radionuclides, particularly U, in the tissues of Beaverlodge fish may have been the cause, although it is unclear whether effects arose via chemo- or radiotoxic routes.

C. Metallothionein

The results of this study indicate U may be a weak inducer of MT in the liver of lake whitefish. More pronounced and consistent induction may occur under other exposure regimes. Metals introduced in the diet may be less effective, or even completely ineffective, at inducing MT in liver and kidney, relative to water-borne metals. Even metals which are potent inducers, such as Zn, have been reported to fail to induce MT when introduced in the diet of fish (Overnell et al. 1988). Furthermore, metal acclimation responses are generally greater in fish that have been exposed to the metal via the water (Miller et al. 1993). It would be of interest to determine the ability of aqueous U to induce MT in freshwater fish, as the response may be more pronounced and persistent than that achieved by the dietary route of exposure.

There are at least five possible explanations for the disappearance of MT induction after day 10 in fish from the high treatment group: (1) the progressive decline in U dosage to fish, (2) overt U toxicity, (3) reproductive influences, (4) the consumption of MT as an antioxidant, and/or (5) acclimation via other pathways and mechanisms.

The first explanation could be adequate if high concentrations of U are required to induce MT. With progressive declines in U dosage accompanying fish growth, a critical minimum concentration necessary to evoke this response may not have been sustained. A second possibility is that MT induction was no longer possible owing to overt U toxicity. Adverse effects in the livers of lake whitefish from this treatment group were observed. Furthermore, both histopathological lesions and reductions of LSI progressed over the exposure period.

It is conceivable that exposure and accumulation of U beyond the tenth day of exposure to high concentrations overwhelmed the MT defense mechanism, perhaps via a reduced capacity for protein synthesis. U may inhibit protein synthesis in mammals (Ghadially et al. 1982) and there is some recent evidence that U stimulates the heat shock protein HSP73 (Mizuno et al. 1997). Because this protein is believed to protect cells from stressor-induced inhibition of rRNA synthesis (Sanders 1990), this induction by U may indicate impaired protein synthesis.

An endogenous factor, such as the reproductive state of the lake whitefish used in this study, could also account for the failure to demonstrate sustained MT induction in U-treated fish. Other than metals, a number of factors and stressors demonstrate some ability to induce MT in teleosts. These include glucocorticoids, such as cortisol (Weber et

al. 1992) and hydrocortisone (Burgess et al. 1993), progesterone (Burgess et al. 1993), noradrenaline (George et al. 1992, Hyllner et al. 1989), antigens (Maage et al. 1990), and various forms of physical stress such as handling (Baer and Thomas 1990), overcrowding (Holdway et al. 1995), starvation (Holdway et al. 1995), and restrainment (Weber et al. 1992). In addition, because Zn is a potent inducer of MT, any condition that disturbs Zn homeostasis and distribution may potentially influence MT concentrations and synthesis (Cosson et al. 1991). In mammals, lipopolysaccharides, interleukin-1, and interferon also induce MT (Hamer 1986, Kagi and Schaeffer 1988).

Furthermore, numerous other variables influence the occurrence and extent of MT induction in fish independently of metal exposure, including reproductive state (Olsson and Kling 1995, Olsson et al. 1987, Overnell et al. 1988), sex (Olsson et al. 1987, Overnell et al. 1988, Schlenk et al. 1996), species (Cope et al. 1994), age (Cope et al. 1994), temperature (Hyllner et al. 1989), and route of metal exposure (Overnell et al. 1988). MT concentrations may also vary considerably among individuals of the same species and population (Cosson et al. 1991, Overnell et al. 1987).

Typically, MT concentrations increase in sexually maturing or spawning fish. This trend is most evident in females but has also been observed in males (Baer and Thomas 1990, Olsson et al. 1987). Because of the vast number of factors known to interact with MT metabolism, Burgess et al. (1993) recently advocated that only sexually immature fish and levels of MT greater than three times reference values should be used as indicators of metal exposure and MT induction. In a recent review, Olsson (1996) emphasized that evaluations of MT induction in fish should avoid the period of sexual

maturation. Thus, the reliability of the present results are questionable because gonadal maturation in lake whitefish may have been associated with endogenous increases in hepatic MT concentrations, independently of U exposure.

Because little base-line biochemical and physiological data exist for the lake whitefish, the 'normal' range of MT concentrations and the capacity for MT induction in the tissues of this species are unknown. Hepatic concentrations of MT measured in this study are approximately twice those found in feral lake whitefish from lakes in northern Saskatchewan impacted by U mining and milling (Klaverkamp et al. 1997). These results indicate that all fish from the present study may have been experiencing MT induction possibly attributable to reproductive cycling.

Furthermore, reportedly metals do not induce MT in fish during periods of vitellogenesis (Olsson and Kling 1995). Thus direct assessment of U's ability to induce MT is confounded here because gonadal maturation may increase MT production and because of the inability of metals to induce MT during the reproductive cycle. The reliability of the current results are also questionable on the basis of unequal sex distributions within and between tanks. Because sex-related differences were observed on day 30 (Appendix one), this non-uniform sex distribution confounds direct inter-group comparisons.

The fourth explanation for the loss of MT induction in fish fed 10 000 µg U/g is related to the role of MT as an antioxidant. In addition to its metal sequestering ability, MT scavenges free radicals that may be generated in the presence of metals (Hamer 1986, Matsubara et al. 1987), thus protecting the cell from oxidative damage. For example, MT

attenuates the adverse effects, particularly lipid peroxidation, arising from Ni-induced free radical generation in mammals, despite the inability of MT to chelate Ni (Srivastava et al. 1995). The potential role of MT as a radical scavenger is further supported by demonstration of its ability to reduce Cd genotoxicity (Coogan et al. 1992) and its ability to inhibit lipid peroxidation (Thomas et al. 1986) in mammals. When functioning as an antioxidant, MT may be consumed and tissue concentrations may decrease. The metabolism of MT in response to augmented oxidative stress is a plausible explanation here as increases in lipid peroxides were observed in the serum of lake whitefish after day 10 and were most notable on day 30 when gonads were largest.

The final possibility, that MT induction was lost due to acclimation via other pathways and mechanisms, is supported by both recent and early studies of mammals. Researchers for the Manhattan Project observed that the acquisition of resistance to U in mammals coincided with some degree of renal repair (Haven 1949, Leggett 1989). Regenerated proximal tubules, reportedly structurally different than their predecessors, were described as tubules with flattened epithelium, decreased brush border surface areas, and reduced numbers of mitochondria. The most common interpretation of these observations was that structural difference equated to functional difference and that this in turn generated acclimation. There is a reasonable theoretical argument to support this hypothesis as a reduction in brush borders may reduce the area available for U attachment, thus reducing U toxicity (Leggett 1989). Conversely, the altered morphology of regenerated tubules is viewed as a pathology by contemporary researchers and not an adaptive response (Leggett 1989).

Because this morphological hypothesis fails to account for the loss of acclimation following discontinuation of U exposure, it appears to be inadequate. It is more plausible that U acclimation arises via a biochemical stress response. A likely candidate is induction of one of the more conventional heat shock proteins (HSP). HSP are of considerable interest as candidates for contaminant biomarkers in wildlife (Dunlap and Matsumura 1997) and aquatic organisms (Livingstone 1993, Sanders 1990, 1993, Stegeman et al. 1992, Triebskorn et al. 1997). The HSP family consists of proteins, named according to size, strongly induced by heat and numerous other physical and chemical stresses, including metals (Caltabiano et al. 1986). Some members are present under non-stressful conditions and function in the maintenance of protein homeostasis. Stress-induced HSP are believed to protect cells from stressor-induced damage, particularly to proteins, and may impart resistance to the stressor (Stegeman et al. 1992).

Two recent publications in the mammalian literature indicate that the acquired resistance of rats to U is achieved via induction of heat shock proteins in kidney. The first study revealed similarities between the resistance of cultured renal cells pre-treated with U or heat to subsequent doses of U (Furuya et al. 1997). The agents quercetin and staurosporine, substances which inhibit the development of thermotolerance, also reduced the resistance of cells pre-treated with U, to a toxic dose of U. These data indicate that the mechanisms by which cells acquire resistance to U are remarkably similar to the development of thermotolerance.

The second study provided more direct evidence of the involvement of HSP in the development of resistance to U. Renal HSP73 increased 148 ± 12% of baseline in rats

injected with high doses (5 mg/kg) of uranyl acetate, concomitant with resistance to rechallenge with the same dose of U and reductions in tubular damage and serum creatinine concentrations (Mizuno et al. 1997). Lower doses (2 mg/kg) of uranyl acetate were less effective in inducing HSP73 and resistance to U. From these studies, the involvement of HSP in the acquisition of resistance to U is highly suspect and certainly warrants further attention in fish.

MT induction may have occurred in lake whitefish tissues other than liver and kidney, in particular, in intestine. The temporal accumulation of U in the intestines of lake whitefish exposed to 10 000 µg U/g food suggests that induction may have occurred. Mean concentrations of U in this tissue were not significantly different between sampling days, indicating an accumulation plateau or equilibrium (see chapter two). This accumulation profile is characteristic of metals which induce and bind to metal-binding proteins, such as MT, explained by the finite capacity for MT induction and synthesis and subsequent saturation of metal binding sites. The ability of other metals to induce MT in fish intestine has been demonstrated, particularly when exposure is achieved via the diet (Handy 1996, Roesijadi 1992). Induction in intestines is adaptive because it impedes the passage of metals into the circulation, and ultimately, their uptake in other tissues (Petering et al. 1990).

One final consideration relevant to interpretation of the results of this study, is related to the methodology of the Hg saturation assay (Dutton et al. 1993, J.F. Klaverkamp, The Freshwater Institute, 501 University Crescent, Winnipeg, Manitoba, Canada, R3T 2N6; personal communication) which was employed for MT estimation.

Although reliable for the measurement of MT in tissues containing Cu, Zn, Ag, Hg, or Cd, this analytical technique may not be the most appropriate for tissues containing U. The premise of this and all other metal displacement assays is the ability of Hg to displace *in situ* metals. This is ensured for Cu, Zn, Cd, and Ag, because of these metals, Hg possesses the greatest affinity for MT metal binding sites (Dutton et al. 1993). In the Hg saturation assay, MT is labelled with ^{203}Hg and concentrations are estimated via gamma counting and from standard curves. However, a metal with a greater affinity for MT binding sites than mercury, may not be displaced and, ultimately, MT concentrations would be underestimated. As the affinity of U for MT metal binding sites is unknown, this scenario remains a possibility.

D. Lipid Peroxidation

U caused a dose-dependent increase in the concentration of lipid peroxides in lake whitefish serum on days 30 and 100. There are two main pathways through which a contaminant may stimulate lipid peroxidation. First, contaminants, particularly metals that exist in more than one oxidation state (Horton and Fairhurst 1987), may enhance free radical production. Metals may function as catalysts in the degradation of lipid hydroperoxides which are normally rather stable. Because metabolism of membrane lipid hydroperoxides generates free radicals, expedition of this process elevates lipid peroxidation (Horton and Fairhurst 1987). Contaminants may also induce free radical generation via stimulation of the P450 monooxygenase system (Phase I enzymes) or via

direct participation in oxido-reduction reactions and electron transfer pathways (Benson and Di Giulio 1992, Di Giulio 1991).

The second major route by which lipid peroxides may be increased by contaminants is via depletion or inhibition of antioxidant defence systems (Di Giulio et al. 1989, Reddy et al. 1981). Several proteins (MT and glutathione) (Di Giulio 1991, Matsubara et al. 1987), vitamins (ascorbate (C) and tocopherol (E)) (Di Giulio 1991), and enzymes (catalase, superoxide dismutase, glutathione peroxidase) (Di Giulio 1991, Di Giulio et al. 1989) function as potent antioxidants due to their ability to sequester and detoxify free radicals.

There is a reasonable theoretical argument in support of the ability of U to behave as a prooxidant. The reduction of U^{6+} to U^{4+} (Voegtlin and Hodge 1949), the two predominant species in biota (Wrenn et al. 1985), could theoretically generate reactive oxygen species by catalyzing the breakdown of lipid hydroperoxides (Di Giulio 1991). Furthermore, U inhibits cytochrome oxidase activities in mammalian kidney and liver (Dounce and Lan 1949, Singer et al. 1951), is highly toxic to cellular and subcellular membranes (Tasat and de Rey 1987), inhibits the activities of cytochrome P450 enzymes (Pasanen et al. 1995), oxidizes ascorbic acid in mammalian kidneys, and stimulates renal catalase activity in chronically poisoned animals (Dounce and Lan 1949). The latter observation is particularly significant because catalase induction is a characteristic adaptive response to oxidative stress (Benson and Di Giulio 1992).

Concentrations of LPO in lake whitefish serum in all three treatment groups were higher on day 30 than day 100. There are several possible explanations for this

observation, including: (1) decreasing U dosage with increasing time, (2) U acclimation, and/or (3) gonadal maturation. Because the first two possibilities have already been discussed at other points in this document, no further explanation is warranted here. The third postulation is discussed below.

Gonadal maturation could directly or indirectly enhance lipid peroxidation. A direct involvement may occur if lipid peroxidation were heightened in this tissue during maturation. Because peak U accumulation in this tissue, as well as gill, coincided with peaks in serum LPO, this appears a likely possibility. Developing gonads contain a high fraction of PUFA (Steffens 1989) which might render them vulnerable to lipid peroxidation and could result in elevations of serum LPO during advanced growth. The high concentration of U observed in gonads on day 30 is in agreement with this notion.

Indirect involvement of gonadal maturation may result from the compounding demands imposed by maturation and stress due to U exposure, which collectively may overwhelm the antioxidant defence mechanisms of fish, hence exacerbating U toxicity. Vitamin C and vitamin E reserves could have been depleted on day 30 owing to their involvement in fish reproduction (Steffens 1989), leaving less available for antioxidant functions. Because salmonids can not synthesize vitamin E and have only a weak ability to synthesize ascorbic acid, they must obtain both in the diet (Steffens 1989). Ascorbic acid and vitamin E requirements peak during gonadal ripening in teleosts.

Vitamin E is elevated in the ovaries of spawning fish where it reduces the level of malondialdehyde dehydrogenase, a measure of lipid peroxidation (Steffens 1989). Increased demands for vitamin E in developing gonads are associated with elevations in

the PUFA content of this tissue. Deficiencies during gonadal maturation cause many alterations in egg quality and gonad condition, including reductions of phospholipids, and reduce the survival rate of eggs and fry (Watanabe 1985). Generally, vitamin E deficiencies cause a number of pathologies in fish, such as: reproductive defects, fatty degeneration of liver, muscular dystrophy, impaired growth, exophthalmia, ascites, lethargy, loss of skin colour, increased mortality, anaemia, and haemolysis (Steffens 1989).

Ascorbic acid concentrations in, and delivery to, fish gonads fluctuate with the reproductive cycle. In a recent study of dab (*Limanda limanda*), the highest concentrations of vitamin C in ovaries occurred just prior to ovarian maturation and declined thereafter until spawning (Saborowski et al. 1997). Similarly, in male dab, the concentrations varied with testes maturation, being lowest during the prespawning and spawning periods. Delivery of ascorbic acid to ovaries was greatest during gonadal maturation and peaked at spawning. In males, peak delivery coincided with testes growth. It was suggested that ascorbic acid is shunted from other organs to meet these increased demands in gonads during reproductive maturation. In ovaries, this increase could serve several functions: enhance collagen formation, enhance deposition of vitamin C in ova, and attenuate lipid peroxidation in rapidly growing and dividing cells of ovaries and embryos. In developing testes, ascorbic acid could also provide protection against lipid peroxidation in dividing cells (spermatogonia, spermatocytes, and spermatids), ultimately enhancing sperm quality.

The latter suggestion was supported by a second recent study of rainbow trout. Like ovaries, spermatozoa contain high fractions of PUFA, and hence are also particularly vulnerable to lipid peroxidation (Liu et al. 1997). Ascorbic acid concentrations peak in the seminal plasma of rainbow trout during the beginning of the reproductive cycle and decline thereafter. This decline is associated with increases in lipid peroxidation. Thus, if U were increasing lipid peroxidation in gonads, perhaps greatest during the maturation period, it would be expected to impair reproductive functioning and reduce the viability of offspring. Furthermore, if ascorbic acid were shunted from other soft tissues to meet increased demands in the maturing gonads of fish, as suggested by Saborowski et al. (1997), this could translate to reduced antioxidant defences in other tissues. This possibility could provide an explanation for the observed peaks in serum lipid peroxides on day 30, when gonads were the most mature.

Many detoxification enzymes and pathways are modulated by the reproductive status of fish and could play a role in determining the degree of secondary stress effects manifested by lake whitefish exposed to U. For instance, hepatic monooxygenase (i.e. mixed-function oxidases or cytochrome P-450's) activities are dependent upon sexual development (Jimenez and Stegeman 1990). During gonadal maturation, males have higher monooxygenase and cytochrome P-450 contents in liver. Oestradiol inhibits total cytochrome P-450 and specific monooxygenase activities.

Conversely, peaks in serum LPO observed on day 30 may be a result of acclimation to U and/or the development of compensatory mechanisms by day 100. Likely candidates include stimulation of antioxidant defence systems or induction of

metal-binding proteins. However, this explanation seems less likely than the former, because the development of acclimation to metals usually occurs quite rapidly and would have been expected prior to day 30.

E. Research Recommendations

Because the toxicology of U is virtually unstudied in aquatic biota, including freshwater fish, there remain many areas of U toxicology to be addressed. U exerts its toxicity in part by stimulating the process of lipid peroxidation in fish (this study) and is a potent genotoxin in mammals (Lin et al. 1993, Prabhavathi et al. 1995), a second pathway of oxidative stress. Thus, it would be of value to further characterize the mechanisms of U toxicity via oxidative stress pathways, including evaluations of genotoxicity, in teleosts. Indicators of genotoxicity are considered to have potential as biomarkers for the hazard assessment of contaminated sediments (Benson and Di Giulio 1992). Furthermore, the tissue(s) experiencing enhanced lipid peroxidation should be identified as this was not addressed in the present study. Identifying the main sites and the mechanisms of U toxicity could provide useful information for developing sensitive biomarkers of U exposure for application to biomonitoring of impacted aquatic systems.

Because evaluations of MT in lake whitefish were confounded by reproductive development, further evaluations of the interaction between U and this stress protein are recommended. Alternatively, the involvement of non-MT stress proteins (i.e. conventional HSP) in the potential development of acclimation to U in fish appears to be a promising line of study, as suggested by contemporary mammalian researchers (Furuya

et al. 1997, Mizuno et al. 1997). Conventional HSP are being developed as biomarkers of contaminant exposure in aquatic organisms (Sanders 1990), and could be pursued with respect to this contaminant.

Histopathology in tissues other than liver and posterior kidney could be evaluated. Fish intestine should be examined as it is a major site of U deposition and the site of uptake and could be structurally damaged by diets contaminated with U. The structural integrity of two of the major accumulation sites, bone and scales, should also be assessed. Skeletal tissues in particular may be damaged by bone-seeking metals in fish (Bengtsson 1974, 1975, Bengtsson et al. 1975, Holcombe et al. 1976, Mehrle et al. 1982, Slooff 1982, Valentine 1975) and there is direct evidence that U alters the structural and functional integrity of mammalian bone (Guglielmotti et al. 1984). Furthermore, recent research indicates that metals may alter the structure and regenerative processes of freshwater fish scales (Rishi and Jain 1998, Yoshitomi et al. 1998).

Finally, many aspects of U toxicology (and accumulation) appear to be affected by gonadal maturation in lake whitefish. Several parameters were most severely or exclusively affected in U-treated fish on sampling day 30, when gonad size was greatest. This evidence indicates a possible heightened sensitivity to U in fish during the reproductive period. This possibility requires further investigation because of the potential for impact upon fish health and reproduction. Parameters that could be evaluated include: endocrine effects, gonadosomatic index, fecundity, egg size, maternal transfer of U, lipid content of ova, lipid peroxides and vitamin C and E in gonads, ova, and gametes, egg morphometrics, and histopathology of gonads, embryos, and fry.

Higher level effects that could be addressed include reproductive behaviour (e.g. spawning success), egg hatching, deformities, and survivability of offspring. Furthermore, it would be of interest to evaluate general indices of health and U toxicity in fish during the reproductive period.

III. Environmental Relevance and Implications

A. Exposure

One of the immediate objectives of this study was to evaluate the potential for U to accumulate in fish, when introduced in the food. Ultimately, the results could be applied to environmental monitoring of feral fish inhabiting contaminated aquatic systems. Thus, some discussion regarding environmental exposure is warranted.

Because lake whitefish obtain a portion of their diets from the sediments, they, as do other benthic feeders, consume the sediments themselves. Thus, there is a direct link between the transfer of U in sediments to fish. As stated earlier, concentrations of U reported in the sediments of systems in northern Saskatchewan that receive U mine and mill discharges range from < 100 µg U/g to > 1000 µg U/g (dw) (Hynes 1990, Hynes et al. 1987, Joshi et al. 1989, McKee et al. 1987, Neame et al. 1982, Swanson 1985). Concentrations as high as 18 000 µg/g (dw) were observed in sediments from Port Hope Harbour, near the site of a U refinery (Hart et al. 1986). These environmental

concentrations are similar to the exposure concentrations employed in the present study, and largely formed the basis for their selection.

U is also transferred to fish via consumption of contaminated diet items, most notably benthos. The diet of lake whitefish inhabiting the Beaverlodge Lake system, northern Saskatchewan, consists primarily of chironomids, lake chub, stickleback, detritus, benthic algae, molluscs, and crustaceans (Swanson 1982, 1983). This is typical of the diets of lake whitefish across Canada (Scott and Crossman 1973). It is well established that U concentrations decrease with increasing trophic level in the order: algae > invertebrates > fish (Mahon 1982). Thus, benthic feeding fish, such as the lake whitefish, are particularly susceptible to U exposure and, hence, accumulate greater concentrations of U than predatory and pelagic feeders (Emery et al. 1981, Kovalsky et al. 1967, Swanson 1982, 1983, 1985). Few studies, however, have addressed the issue of U accumulation in fish diet items, such as forage fish, benthic macroinvertebrates, and, particularly, benthic algae.

In one of the scarce reports of U residues in fish diet items, relatively low concentrations were observed. U residues (mean ± SE) in insects collected from a U mill tailings system which drains into Beaverlodge Lake, Saskatchewan, ranged from 2.64 ± 0.47 μg U/g (ww) in blackflies (*Simulium* sp.) to 25.63 ± 2.59 μg U/g (ww) in caddisflies (*Nemotaulius* sp.) (Swanson 1985). Forage fish contained concentrations of U ranging from 0.52 ± 0.30 μg/g (ww) in Beaverlodge Lake to 3.39 ± 0.78 μg/g (ww) in the tailings system.

Other data indicate that benthic organisms accumulate high concentrations of U relative to the sediments. Benthic biota, primarily *Limnodrilus hoffmeisteri* and *Tubifex tubifex*, inhabiting Port Hope Harbour, Lake Ontario contained between 1.7 and 40.9 µg U/g (dw) when sampled in 1984 (animals were held for 24 h in the lab to purge the gut contents) (Hart et al. 1986). At this time, concentrations of U in surficial sediments were low (92.5 µg/g) relative to earlier concentrations (18 000 µg/g in 1968), largely attributed to decreased U discharges, although repeated dredging of the sediments may have also contributed to these reductions.

Evaluations of U residues in the biota inhabiting aquatic systems impacted by U mining and milling in northern Saskatchewan that have not included benthic algae, may have underestimated the potential exposure of fish to U. In general, phytoplankton and littoral algae accumulate the highest concentrations of U in the aquatic trophic web (Wahlgren et al. 1976). Thus, benthic, as well as pelagic, algae may be an appreciable source of U to fish. For instance, in a lake naturally enriched in U, the algae *Chara* sp. contained U at concentrations one and a half times greater than those found in the underlying sediments (Koval'skiy and Vorotnitskaya 1965). Similarly, algae and sediments, collected from an aquatic system located in an uraniferous region of the Okanagan Highlands, British Columbia, contained similar concentrations of U (Mahon 1982). This relationship was also demonstrated in a system impacted by U mining. U concentrations in green algae (*Spirogira*) were higher than in sediments sampled from a river receiving U mine water discharges in Slovenia (Stegnar and Kobal 1982).

U also displays a strong tendency to accumulate in and on organic matter (Kalin 1988), such as detritus. This attribute is illustrated by the high degree of correlation between U concentrations and organic matter contents of sediments (Koval'skiy and Vorotnitskaya 1965). Furthermore, as U is believed to be primarily adsorbed to the surface of organic detritus (Kalin 1988), microorganisms (Yong and Macaskie 1995), and algae (Fisher et al. 1987), rather than assimilated within the tissues, U may be even more bioavailable to the consumer than U which is directly incorporated into living biomass.

Another important factor contributing to exposures of feral fish to U is the presence of U in the water column. The present study was designed to evaluate the dietary route of exposure and did not attempt to address the issue of aqueous exposure. However, concentrations of waterborne U are generally elevated in aquatic ecosystems impacted by U mining and milling (e.g. Hynes 1990, Swanson 1985). U exists in oxic waters in the hexavalent oxidation state (Choppin and Stout 1989), the most bioavailable form of U (Sullivan et al. 1986, Tannenbaum 1951). Because U is highly soluble in oxic surface waters (Cowart and Burnett 1994), it is often present at relatively high concentrations in aquatic systems impacted by U mining and milling (Hyne et al. 1992b, Hynes 1990, Hynes et al. 1987, Kalin 1988, Moffett and Tellier 1978, Nichols and Scholz 1989, Parkhurst et al. 1984, Swanson 1983, 1985) and may be a significant source of exposure in some systems.

For example, U concentrations in surface waters within tailings systems have reached values as high as 39.8 mg/L in the Elliot Lake region, Ontario (Moffett and Tellier 1978) and 4.3 mg/L in the Beaverlodge area, Saskatchewan (Swanson 1985).

Within receiving water bodies, such as Beaverlodge Lake, mean ± SE concentrations of U in surface waters were 338 ± 193 µg/L, shortly before decommissioning of the mill (Swanson 1985). Over 15 years following the closure of this mining and milling operation, concentrations of U in surface waters at several sampling sites were in excess of 1 mg/L; over four times the close-out water quality objectives for this contaminant (Cameco 1997). At some sites the daughter nuclide, ^{226}Ra, has been steadily increasing in concentration since the closure of this operation.

U concentrations in the surface waters of Island Lake, which receives effluents from the Cluff Lake U mine and mill, had increased approximately 1100 times above the baseline following only 6 years of effluent discharges (Hynes 1990). Concentrations were 774.9 ± 128.0 µg/L (mean ± SD) following only 2 years of effluent discharges, and averaged 527.0 ± 232.1 µg/L (mean ± SD) over 6 years (Hynes 1990). These values are considerably higher than values reported as background, 0.45 µg/L (Kalin 1988), and the acceptable concentration (100 µg/L) in drinking water (Health and Welfare Canada 1993).

For comparison, Langan (1983) recommended that a concentration of 300 µg U/L in surface waters is acceptable for the protection of aquatic life. Concentrations of aqueous U that are acutely toxic to cold-water and warm-water fish (Appendix four) are generally below concentrations observed around Elliot Lake, however, this region represents a case of an exceptionally high degree of pollution. Typically, acutely toxic concentrations of U to fish are above this suggested limit and higher than concentrations observed in surface waters contaminated by U mining and milling. The lowest reported

LC50 (96-h) for cold-water species (2.8 mg/L) was observed in the fathead minnow (*Pimephales promelas*) in softwater (Parkhurst et al. 1984).

Conceivably the concentrations of U occurring in polluted surface waters could exert sub-lethal toxicity in fish and would be expected to contribute to U exposure and accumulation in fish residing in these systems. However, there are currently no water or sediment quality guidelines for U aimed at protecting non-human biota. Australia, currently undergoing substantial U mining developments, has set an interim guideline of only 5 µg U/L for water bodies receiving U discharges (Bywater et al. 1991). Similarly, Bywater et al. (1991) estimated a no-observed-effect-concentration of 16 µg U/L for the protection of tropical freshwater fish and cladocerans. It is noted that warm-water fish and cladocerans appear to exhibit greater sensitivities to U than their cold-water counterparts (Appendix four, Bywater et al. 1991, Vinot and Larpent 1984). However, when the hardness of the test water is similar, warm- and cold-water fish and cladocerans exhibit equal sensitivities to acutely toxic concentrations of U (Bywater et al. 1991). Therefore, the strict standards imposed by the Australian government and the advocations of Bywater et al. (1991) lead one to question the safety of U concentrations observed in polluted surface waters in Canada and the absence of environmental guidelines in this country.

When assessing U exposure and potential hazards to feral fish, it is also important to consider interactions between U accumulation and parameters such as fish nutritional status, food availability, quality, and abundance, and the chemical characteristics of the aquatic system. Reduced food abundance or availability could augment U accumulation

in fish because fasted organisms may accumulate significantly greater quantities of metals than fed animals. For example, U absorption was doubled and retention increased fourfold in fasted rats, relative to fed rats (Sullivan et al. 1986). Similarly, U was excreted three times faster in fed crabs (*Pachygrapsus laevimanus*) than in fasted crabs, resulting in a significant reduction in U accumulation in the former (Ahsanullah and Williams 1989). Greater accumulation and retention of Cu has also been observed in starved, relative to fed, fish (Segner 1987). Furthermore, dietary iron deficiencies, or manifested in the organisms themselves, may heighten U accumulation via the diet, as has been observed in mammals (Sullivan and Ruemmler 1988).

The transfer of metals from aquatic sediments and lower trophic organisms to fish is further influenced by indirect effects of the contaminants on fish diet items. Reductions in species diversity with subsequent increased dominance of tolerant organisms, or complete replacement of sensitive taxa, will impact upon the quality of fish diets. Furthermore, tolerant organisms may accumulate higher concentrations of metals than more susceptible species (Dallinger and Kautzky 1985), thus augmenting exposure of fish to these metals. This positive feedback pathway intensifies exposure of fish to metals in polluted habitats (Dallinger et al. 1987).

Concentrations of U in water at or below those observed in impacted systems have been shown to produce adverse effects in invertebrates exposed in the lab. Solutions containing 2 mg U/L depressed the growth of the marine amphipod (*Allorchestes compressa*) exposed for four weeks (Ahsanullah and Williams 1986). At 1 mg U/L, effects were seen in the progeny, including reductions in the number of males, the sex

ratio, and respiration rate. A concentration of 200 µg U/L, the lowest examined, inhibited population growth in the freshwater hydra in only three days (Hyne et al. 1992b). Similar sensitivity was reported for *Daphnia magna*; concentrations between 0.5 and 3.5 mg U/L suppressed reproduction and increased mortality (Poston et al. 1984). The 24-h LC50's for warm-water cladocerans are as low as 410 µg/L (Bywater et al. 1991).

If fish food and/or nutrients are limited in quantity and/or quality in U-enriched aquatic systems, fish may accumulate more U than in more favourable environments. In the event that fish diet items are adversely affected by the contaminants in systems impacted by U mining and milling, particularly where abundances are reduced, ultimately, resident fish would be threatened by both nutritional stress as well as by heightened U accumulation.

Consequently, the present study may be considered conservative with regards to evaluating exposure typical in the natural environment, because feral fish are exposed to U via both the diet and water. Due to the paucity of data on U concentrations in aquatic biota, it is difficult at present to ascertain the precise exposure regime (i.e. U concentrations) to which feral fish are subjected. Surveys within the natural environment and additional studies of exposures at lower concentrations for longer durations in a controlled environment are required.

B. Effects

U exerted sub-lethal toxicity in lake whitefish fed diets containing U at concentrations as low as 100 µg/g. As these concentrations are typically observed in

surficial sediments of Canadian freshwater ecosystems receiving U mining and milling discharges (Appendix two), this observation is highly environmentally relevant. Further evaluations of the effects of U in freshwater fish exposed to lower concentrations for longer periods would aid considerably in establishing environmental criteria.

A number of parameters investigated in this study were most severely or exclusively affected in lake whitefish when gonadal development was greatest. Alterations exclusively observed at this time were elevations in serum chloride and osmolality. Effects that were most pronounced at this time were glomerular lesions and serum lipid peroxides. Accumulation of U in gonads and gills were also greatest when gonads were largest.

Based on these data, it appears that fish may be most profoundly affected by U during the reproductive period and that accumulation in gonads may be heightened during periods of gonadal growth. As these observations may have significant implications for fish health and reproduction, further research on the interactions between U and fish reproduction is highly recommended.

C. Relationship Between U Tissue Residues & Toxicity: Potential Biomarkers

U residues in intestine, kidney, liver, bone, and scales proved the most sensitive and reliable indicators of U exposure. Gonads accumulated high concentrations of U in fish fed the highest test diet, most notably during peak gonadal development. U toxicity was most reliably demonstrated by the occurrence of histopathological lesions in liver and posterior kidney and by elevations in serum lipid peroxides. The inclusion of these

analyses in biomonitoring programs designed to evaluate the bioavailability of U to, and health of, feral fish inhabiting impacted systems is recommended.

Clear relationships between concentrations of U in lake whitefish tissues and adverse effects could not be established owing to the low sensitivity of the instrument used for U analysis. However, results indicate that concentrations of U in kidney and liver associated with adverse effects in lake whitefish were below the tissue detection limits of 3.13 µg U/g and 2.08 µg U/g, respectively.

A concentration of 3 µg U/g in mammalian kidney was deemed the critical threshold of chemical toxicity by the ICRP in 1959, and remains in general acceptance (Spoor and Hursh 1973). However, because a number of researchers have revealed significant damage in kidneys containing less U, there have been suggestions to decrease this value to 1 µg U/g (Diamond 1989, Leggett 1989, Morris and Meinhold 1995, Wrenn et al. 1985).

Langan (1983) concluded that fish are less sensitive to the chemotoxicity of U than are mammals. On this basis, it is reasonable to assume that the critical threshold concentration of 3 µg U/g in mammalian kidney should be conservative when applied to teleosts. In the present study, significant damage was observed in the kidneys of lake whitefish fed all three concentrations of U, 100, 1000, and 10 000 µg U/g food, for 100 days. Detailed descriptions of these effects are provided in chapter three. It is relevant to note here that renal histopathologies were observed in lake whitefish where U could not be detected in this tissue. However, the analytical detection limit for lake whitefish kidney, 3.13 µg U/g ww, is close to the suggested critical threshold concentration of 3 µg

U/g kidney in mammals. Thus, while it is not possible to comment upon the precise relationship between U concentration and "effects" in fish kidney below this critical value, concentrations of U lower than 3.13 µg U/g in kidney were associated with tissue damage.

These data indicate that fish may be more, or equally as, sensitive to the chemical toxicity of U in kidney as mammals. This is in direct conflict with the current paradigm regarding the relative sensitivities of these two groups (Langan 1983) and warrants additional attention. Evaluations of the critical threshold concentration of U in fish kidneys should be further investigated using more sensitive analytical techniques, longer duration of studies, and analyses of natural fish populations in contaminated habitats.

The author is aware of only two studies which evaluated histopathology in fish inhabiting environments containing above background concentrations of U in sediments. The first study, which examined fish inhabiting Beaverlodge Lake, northern Saskatchewan, was restricted to evaluations of skin, gill, and eye (Bernstein and Swanson 1989). In the second, no lesions attributable to contaminants were found in muscle, intestine, pancreas, vertebrae, or kidney of lake whitefish captured in Langley Bay, Lake Athabasca, Saskatchewan (Waite et al. 1990). Although sediments and fish in Langley Bay contained elevated concentrations of U, the absence of histopathologies are not unexpected as concentrations in tissues were very low; ranging from 0.05 µg/g (ww) in muscle and testis to a maximum of 1.7 µg/g (ww) in bone (Waite et al. 1988). Concentrations in kidney averaged 0.6 µg/g (ww). Effects would be further unanticipated

because Langley Bay is located on a large lake and fish, being highly mobile, would be expected to range out of the study area.

Evaluations of other effects in feral fish from these two contaminated systems lend some insight into interpreting critical concentrations of U in tissues associated with adverse effects in lake whitefish from this study. Fish inhabiting Beaverlodge Lake, a system more heavily contaminated than Langley Bay, displayed some haematological alterations (Bernstein and Swanson 1989). Packed cell volume, total protein, and red blood cell counts were reduced in white sucker and lake whitefish, and white blood cell counts (WBC) elevated in the former species, from Beaverlodge Lake, relative to fish from two reference lakes. Increased WBC of lake whitefish were significant relative to fish from only one of the two reference systems. Less dramatic effects, and the opposite of those seen in lake whitefish and white suckers, were observed in lake trout. Lake whitefish and white suckers from Beaverlodge Lake contained U at concentrations 3 to 115 times greater than those found in reference fish. In the study of Langley Bay, no alterations were found in the following parameters measured in lake whitefish: parasite load, haematocrit, condition factor, fork length, and histopathology, as discussed above (Waite et al. 1990).

The present results and those of Waite et al. (1988, 1990) indicate that the critical concentration of U in lake whitefish kidneys associated with histopathological effects in this tissue likely lies between 0.6 µg/g (ww) and 3.13 µg/g (ww). However, further evaluations of this relationship are required.

Collectively, results of U analyses in fish tissues and indices of toxicity from this study indicate that assessments of liver and kidney histopathology, lipid peroxides, and U concentrations in scales are the most sensitive indicators of U exposure and sub-lethal toxicity. However, residue analyses in other tissues may prove more reliable using more sensitive analytical techniques than those employed here. For this reason, it is strongly advocated that U be analyzed in liver, kidney, intestine, bone, and gonad, in addition to scales, in biomonitoring programs of contaminated habitats.

IV. α-Radiation From Internally Deposited U

A. Results

The calculated annual doses of α-radiation emitted by U deposited in the nine tissues of whitefish at 10, 30, and 100 days of exposure are presented in Appendix five. Data are expressed as annual dose rates (mean ± SE), calculated using tissue concentrations of U measured on the three sampling days. Cumulative doses within the 100 days of exposure are minimal, with the possible exception of intestines of fish fed 10 000 μg U/g food. The relative dose rates to the nine tissues of lake whitefish follow the same order discussed for U accumulation. Thus, the highest doses are in the intestines of fish fed the highest concentration of U and the lowest in fish muscle.

A. Discussion

As presented in Appendix five, the alpha radiation doses emitted by U in the tissues of lake whitefish may be considered negligible over the course of the study. The one exception may be the intestine, particularly in the highest treatment group, in which doses are considerably greater than all other tissues. The biological relevance of the calculated annual dose rates in whitefish arising from internally deposited U are largely unknown. Due to the scarcity of data regarding the effects of internal ionizing radiation in fish, interpreting these results is difficult and is associated with a great deal of uncertainty. The following discussion is an attempt to evaluate the potential chronic radiological hazards associated with internally deposited U in fish.

Alpha radiation, the principle type emitted by internally deposited U, is the most biologically effective form due to its short penetration distance. Because of the small volume of tissue impacted by alpha particles, the same sites within a tissue receiving high linear energy transfer (LET) radiation may be 'hit' (i.e. energy transfer from radiation) multiple times, and thus absorb significantly more radiation than distant sites hit only once (Lang et al. 1995). It is generally accepted that this sensitive volume of tissue for alpha radiation is approximately 1 to 5 μm (Cohen 1980), although α-particles may travel up to 100 μm in biological tissues (Lang et al. 1995). Cells immediately adjacent to an alpha particle would largely be killed as a result of the radiation concentrated in a small volume (Lang et al. 1995).

Furthermore, one existing paradigm for radiation-induced cancer is based upon a 'multi-hit' hypothesis. In this theory, the risk associated with a given target developing a malignancy is proportional to the number of hits it incurs within a short period of time

(Cohen 1980). It is largely believed that multiple collisions close together in time are more effective at inducing cancer, because there is insufficient time for damage incurred from the first hit to be repaired before the second hit is received. Thus, because alpha particles that travel only a short distance are more likely to hit the same target multiple times in a short time span, the risk of developing cancer is much greater than from other sources of radiation (Cohen 1980).

Because the types of radiation possess varying degrees of effectiveness in biological systems, weighting factors are applied. For alpha radiation, the mammalian weighting factor is generally accepted as a value of 20, the highest of all forms of radiation. However, this weighting factor may underestimate the risks associated with low doses of alpha radiation. Some evidence indicates that the adverse effects of alpha particles actually increase at lower dose rates (Cohen 1980). Similarly, there is evidence to suggest the risk of developing cancer per unit dose of external radiation is also increased at low doses (Kneale et al. 1983). Due to the paucity of data on the effects of ionizing radiation in fish, weighting factors are not used. Thus, throughout the following discussion, conversions of weighted radiation doses (i.e. in Sv) to non-weighted units (i.e. rads) in mammals are made without consideration of weighting factors.

There are currently no radiological criteria for the protection of biota other than humans. In humans, an estimated 4.4% increase in cancers and 1.8% increase in birth defects are expected from 91 mSv delivered to the whole body over 30 years (Eisler 1994). This is equivalent to 91 rads (without considering the radiation weighting factor), or approximately 90 times the annual effective radiation dose to the general public.

Limits for the general population are < 1.7 mSv/yr (as an average), equivalent to approximately 1.7 rads/yr, ignoring weighting factors. Some restrictions have been placed upon the maximum permissible dose to certain tissues and body parts of occupationally exposed individuals. These include: skin, hands, forearms, eye lenses, and 'other organs'. This latter category has been assigned an annual limit of 150 mSv/yr (150 rads/yr) and a 3 month limit of 50 mSv (50 rads). The acceptable dose to the whole body of occupationally exposed individuals, 50 mSv/yr (50 rads/yr), is considerably greater than standards for the general public, but is nonetheless relatively low.

It is widely accepted that fish are inherently more tolerant to radiation than mammals (Eisler 1994, Rose 1992). Thus, critical doses associated with the development of adverse effects in mammals should be conservative when applied to teleosts. The prevailing notion accepts that because mammals are the most radiosensitive group, protecting human health should inherently protect the less sensitive taxa. It should be cautioned, however, as others have pointed out (Rose 1992, Swanson 1987), that there is insufficient information on the effects of radiation in fish and other 'lower organisms' to empirically accept this sweeping statement. The literature base on radiation effects in fish is extremely limited both in quantity and in quality and much of this research was generated decades ago with little contribution from contemporary research.

The majority of studies, in the both laboratory and field, that have examined interactions between radiation and fish involve non-ionizing or low LET forms, such as x- and gamma rays. Furthermore, the bulk of the literature discusses effects arising from very high doses delivered over short exposure periods, largely administered by external

routes. These exposure regimes are not representative of the vast majority of chronic environmental exposures and often represent exposure that is associated with the least risk of adverse effects (i.e. low LET, external radiation).

Although U is only a weak radio-emitter and is therefore primarily chemotoxic, chronic exposure to U may be of radiological concern (Wrenn et al. 1985). The ICRP, and other U researchers, have accepted that the critical radiological risk associated with chronic U exposure in mammals, is the development of osteosarcoma (Lloyd et al. 1996, Mays et al. 1985, Stevens et al. 1980, Wrenn et al. 1985). Although the current study was designed to address relatively short-term chemotoxic effects of U, it is recognized that there may be long-term radiological hazards associated with U accumulation, in addition to those produced by chemotoxic mechanisms. In the proceeding section, the biological relevance of alpha radiation in lake whitefish is discussed.

i. Somatic Effects of Radiation in Fish

Fish are deemed the most sensitive aquatic organisms to radiation (Templeton 1980) and early life cycle stages are considerably more sensitive to radiation than adults (Templeton 1980). For example, the acute radiation LD50 for rainbow trout gametes is approximately 50 rads (Welander 1954) and for eyed eggs is approximately 400 to 900 rads (Bonham and Welander 1963). In contrast, the LD50 for adult rainbow trout exposed to external radiation for 30 days is approximately 1500 rads (Rice and Baptist 1974). Early developmental stages of fish show a progressive decline in sensitivity to lethal doses of external radiation, with increasing development (Bonham and Welander 1963).

The most common endpoint examined in radiation-exposed fish is death. However, high-level endpoints, such as death, are not appropriate for assessing the health of fish exposed to radiation in the natural environment. For example, a population of mosquitofish exposed to an external dose rate of 0.22 Gy/yr (equivalent to approximately 22 rads/yr) was apparently 'thriving', and no effects on fecundity were found (Trabalka and Allen 1977). However, adverse effects of radiation were present in these fish as an increase in the frequency of deleterious and recessive lethal genes in the gene pool (Blaylock and Frank 1980). A population of channel catfish inhabiting the Chernobyl Nuclear Power Plant cooling pond, which received doses of radiation of 200-300 rads/d from the sediments and an unreported dose from accumulated cesium, was described as 'thriving' and 'vast', years after the accident in 1986 (Sugg et al. 1996). However, evaluations at lower levels of biological organization revealed significant adverse effects attributable to radiation, specifically, DNA damage.

Furthermore, the effects of radiation appear to be biphasic in fish, and other animals. At low doses, external radiation may be 'beneficial' as it may stimulate growth in aquatic organisms (Cooley and Miller 1971). However, dose rates associated with enhanced growth in fish are somewhat variable. A dose rate of 72 Gy/yr (equivalent to 7200 rads/yr) increased growth of the larval pinfish (*Lagodon rhomboides*) exposed to aqueous ^{65}Zn (Rose 1992). This is in contrast to an acute growth stimulatory dose of only 0.2 Gy (20 rads) reported for salmon (*Oncorhynchus tshawytscha*) and rainbow trout (*S. gairdneri*) (Rose 1992).

Sub-lethal effects of radiation have been frequently characterized in early life cycle stages of fish because they are the most sensitive. Decreased hatching of the eggs of fathead minnow was observed following a cumulative dose of 2.7 Gy (270 rads) emitted by ^{232}U in the water (Rose 1992). An increase in abnormalities was detected in this same species exposed to waterborne ^{238}Pu at doses as low as 5.68 Gy (568 rads) (Templeton 1980). However, the chemical toxicity of U may have contributed to the deleterious effects observed in the former study.

Available data indicate that the most radio-sensitive tissues in adult fish are gonads, blood cells, and haematopoietic tissues. High doses of external radiation may rapidly alter haematological profiles, resulting in decreased red and white cell counts, packed cell volume, and haemoglobin (Patel and Patel 1979). Structural damage has been induced in spleen, kidney, and thymus by acute external doses of radiation (Cosgrove et al. 1975, Woodhead and Setlow 1980). A single dose of 1000 rads rapidly (hours to days) reduced haematopoiesis in the kidneys and spleen, the abundance of lymphocytes in kidney, spleen and thymus, and red and white blood cell counts of the Amazon molly (*Poecilia formosa*) (Woodhead and Setlow 1980). A dose of 500 rads caused severe haematopoietic atrophy in kidney, spleen, and thymus of channel catfish (*Ictalurus punctatus*), persisting until 29 days post-exposure (Cosgrove et al. 1975).

The gut of teleosts may be a target for radiation-induced lesions. The amazon molly exposed to 1000 rads externally displayed a number of histopathological alterations, primarily in the anterior and mid-region of the intestine. Although structural damage was seen as early as 6 hours post-exposure, recovery was virtually complete in

only 6 days (Woodhead and Setlow 1980). Gut histopathologies were evident in juvenile rainbow trout (O. mykiss) fed diets containing (370 000 Bq ^{65}Zn/kg/d or 18.5 million Bq ^{90}Sr-^{90}Y/kg/d for up to 17 and 21 weeks, respectively (Eisler 1994).

Other reported effects of exposure to high doses of radiation include: immune suppression, hatching abnormalities, developmental abnormalities, decreased growth rates, decreased fecundity, ovarian damage, mutations in sperm, spermatids, and spermatogonia, and sterility (Eisler 1994). The main tissues of radiological concern in the present study are intestines, bone, and scales, due to their high ability to accumulate U, and kidney and gonads, due to their high radio-sensitivities and moderate accumulation potentials. The biological relevance of alpha emissions from U accumulated in these tissues is discussed below.

Kidney

Like mammals, the most profound changes are manifested in fish erythrocytes, leucocytes and haematopoietic tissues. As opposed to mammals, fish leucocytes are produced primarily by kidney and spleen. The threshold radiation dose in carp (*Cyprinus carpio*) kidney associated with adverse alterations in leucocyte numbers and phagocytic activity was estimated at 0.5 to 1.0 mGy/d or a cumulative dose of 0.04 to 0.05 Gy (Swanson 1987). This is equivalent to approximately 50 to 100 mrad/d, 18.3 to 36.5 rads/yr, or a cumulative dose of 4 to 5 rads.

Although white suckers and lake whitefish from Beaverlodge Lake, Saskatchewan displayed haematological alterations (Bernstein and Swanson 1989), it is not known

whether these lesions are attributable to radiation, chemical toxicity, or interactions between the two. Total dose rates to these fish were estimated at 0.03 to 0.05 mGy/d, 3 to 5 mrads/d or a total of 1 to 2 rads/yr, for a period of 30 years. This amounts to a cumulative dose of 32.9 to 54.8 rads. The main effects observed were decreased red blood cell counts, total protein, and packed cell volumes, while white blood cell counts were elevated above those found in reference fish (Bernstein and Swanson 1989). At the population level, the fecundities and growth rates of Beaverlodge Lake fish were decreased, relative to fish from reference systems (Swanson 1982, 1985). However, high level effects (i.e. growth and fecundity) could not be directly attributed to radiation exposure, but rather, may have resulted from differences in food supply and habitat availability between this system and the reference lakes (Swanson 1982). Unfortunately, the kidneys of these fish were not examined for structural or functional alterations.

The highest mean (± SE) annual dose rate in the kidneys of lake whitefish fed a contaminated diet was calculated to be 430 ± 77 mrad/yr, arising from a concentration of 16.5 ● 3.0 µg U/g kidney, in fish fed 10 000 µg U/g food for 100 days. This is two orders of magnitude lower than the threshold dose of 18.3 to 36.5 rads/yr associated with adverse haematological effects in carp (Swanson 1987). However, the estimated cumulative threshold dose of 4 to 5 rads in carp (Swanson 1987) would be realized in lake whitefish following 9.3 years of exposure to 10 000 µg U/g, assuming this tissue concentration was maintained. Because this time period is within the longevity of lake whitefish (Scott and Crossman 1973), the maintenance of U in kidney at or near the

concentrations measured on day 100, could pose a significant long-term health risk to feral fish.

Intestine

Although little is known regarding the effects of ionizing radiation on fish gastrointestinal tracts, it is generally accepted that this tissue is relatively susceptible to radiation damage in mammals due to its inherent high rate of cell proliferation (Lang et al. 1995). Single external doses of radiation (2000 to 3000 rads) damaged the gastrointestinal capillary system and the gastroepithelium of bluegills (Eisler 1994). Similarly, intestinal damage was induced in the amazon molly exposed to 1000 rads of external radiation at three months of age (Woodhead and Setlow 1980). Among the deleterious effects observed in these studies were excessive epithelial sloughing, epithelial pyknosis, and haemorrhaging (Eisler 1994, Woodhead and Setlow 1980). The suggested critical dose, 30 Gy (3000 rads), for the development of acute mucositis in the small intestines of mammals (Silini 1983) is high relative to critical doses in other mammalian tissues and doses shown to damage the intestines of fish.

Mean radiation doses from U accumulated in the intestines of fish are negligible, with one possible exception. Because the intestines of fish fed 10 000 µg U/g accumulated high levels of U, they received the highest radiation dose rates in all tissues examined. Dose rates in this group ranged from 12.5 ± 5.9 rads/yr on day 10 to 22.9 ± 9.6 rads/yr on day 100. No information is available to directly gauge the biological relevance

of these levels of radiation in this tissue of fish, as the studies outlined above used acute single doses of radiation administered by external routes.

The threshold radiation doses in carp kidney associated with adverse haematological alterations were estimated to be 50 to 100 mrad/d, 18.3 to 36.5 rads/yr, or a cumulative dose of 4 to 5 rads (Swanson 1987). If the intestines are equally as sensitive as the kidneys of fish, then annual dose rates of 12.4 to 22.9 rads/yr may constitute a significant radiation hazard.

Furthermore, the critical cumulative dose of 4 to 5 rads in carp kidney (Swanson 1987) is achieved in the intestines of lake whitefish fed 10 000 μg U/g within the experimental exposure period. By day 100, the intestines of these fish had received a cumulative dose of approximately 5.53 rads from U incorporated into the tissue, ignoring radiation emitted from U in food and faeces. Long-term radiation effects in intestine would not be unexpected at these dose rates.

Gonads

Because gonads are radio-sensitive and accumulated high levels of U in some lake whitefish, a discussion of dosimetry in this tissue is warranted. Data on the critical doses of internally deposited radionuclides associated with damage to fish gonads are extremely limited. The majority of the literature pertains to the effects of external x- and γ-radiation, usually administered at high levels. The types of effects caused by the latter exposure regimes range from structural damage to ovaries and testes and reduced egg or sperm production, to sterility, sex reversal, hermaphrodism, and reproductive failure. Of the two

sexes, the evidence indicates that testes are more sensitive than ovaries (Woodhead et al. 1983).

Available data indicate that relatively low doses of external radiation may cause structural and mutagenic effects in teleost gonads. For example, single exposures to 64, 475, and 950 rads caused a dose-dependent increase in total mutations in the sperm, spermatids, and spermatogonia of adult male medaka (*Oryzias latipes*), mutations that were dominant lethals (Shima and Shimada 1991). Spawning male sea lamprey (*Petromyzon marinus*) that survived a single dose of 2000 rads were sterilized (Hanson 1990). Doses of only 50 to 100 rads to the gametes of rainbow trout (*O. mykiss*) were sufficient to cause a 50% reduction in fecundity (Donaldson and Foster 1957). Furthermore, radionuclides incorporated into the developing embryos of fish cause increases in the frequency of nuclear disruptions (scorpionfish and turbot) (Eisler 1994).

A unique opportunity to study the chronic effects of radiation on fish residing in the natural environment was provided by the horrific disaster that befell the Ukraine, and the global environment, on April 26, 1986. Investigations into the health of fish residing in the cooling pond of the Chernobyl Nuclear Power Plant determined that, although fish persisted in this heavily contaminated system, they suffered from a number of reproductive and developmental abnormalities (Verigin et al. 1996). Silver carp (*Hypophthalmichthys molitrix)* that were 1 to 2 years old at the time of greatest radiation impact (1989-1992) developed sterility, bisexuality, and morphological alterations of gonads and oocytes. Cumulative doses to the fish surviving the accident ranged from 7 to 8 Gy (700 to 800 rads) in 1989 and increased to 10 to 11 Gy by 1992 (1000 to 1100 rads).

The reproductive systems of adults of both sexes and their offspring were adversely affected. Alterations in females who survived the accident included enlarged hypophyses in sterile individuals, relative to spawning individuals and fish from a reference fish farm (Verigin et al. 1996). Radiation-induced lesions in males included: decreased sperm ejaculate volumes and sperm concentrations, increased frequencies of individuals with low spermatozoa concentrations, and histopathological alterations of gonads. Abnormalities observed in offspring of these survivors included a high incidence of sterility, bisexuality, morphological alterations of previtellogenous oocytes, fibrosis of male gonads, and a decrease in male sexual cells (Verigin et al. 1996).

The highest mean dose rate to gonads, produced by U decay, was seen on day 30, the time at which accumulation peaked and at which point fish gonads were the most mature. The calculated dose rate to the gonads of fish fed 10 000 µg U/g food for 30 days was 3220 ± 1332 mrad/yr, based upon concentrations measured on this sampling day. The estimated mean dose to fish from this same treatment group on the 100th day of exposure was 1229 ± 517 mrad/yr. Because heightened accumulation of U appears to be associated with gonadal maturation, radiation doses to fish gonads would be expected to fluctuate with season and spawning condition.

In comparing dose rates on days 30 and 100 to the literature, no immediate effects on fish gonads would be anticipated. However, if the concentrations of U measured in the gonads of lake whitefish from this study were achieved and maintained in feral fish over their life-span, radiological risks would be significant. It would take 15.5 to 40.6 years of continued exposure to dose rates between 3220 mrad/yr and 1229 mrad/yr, for fish to

receive a critical cumulative dose of 50 rads (Donaldson and Foster 1957). The longevity of lake whitefish may be within the estimated 15.5 years required to reach this critical dose (Scott and Crossman 1973).

The embryos of fish residing in the natural environment would be simultaneously exposed to internal and external radiation, particularly in those species which deposit eggs on sediments, a scenario which may augment dose rates. U was accumulated by the eggs of carp exposed to ^{233}U in the water, primarily within the egg contents (~60%) and less in the chorion (Pentreath and Fowler 1979). Therefore any U deposited in the eggs during oogenesis may be supplemented by accumulation from water, and potentially, sediments. Thus, in the natural environment there is a risk that the developing young of fish could be exposed to critical doses of radiation due to U exposure.

With the preponderance of data regarding the effects of alpha radiation emitted by internally deposited radionuclides in fish, it is difficult to reliably assess the radiological risks associated with U accumulation in the present case. Furthermore, although U may contribute a significant portion of radiation dose to aquatic biota, systems contaminated with U generally contain elevations in U daughter progeny which themselves are much more potent radiation emitters. Thus, in the natural environment, the vast majority of radiation doses received by fish originate from transuranics. However, the relatively low activity of U should not preclude its inclusion in radiological assessments of contaminated environments. Further research on the effects of internal radiation in lower vertebrates is required in order to ascertain the risks associated with U deposited in the soft tissues of fish.

ii. Radiation and Carcinogenicity: Bone and Scales

While the main target tissue for the chemotoxic action of U in mammals is the kidney (short-term effect), the site most vulnerable to the radiotoxic action of U is the bone (chronic effect) (Lloyd et al. 1996, Mays et al. 1985, Stevens et al. 1980, Wrenn et al. 1985). In mammals, bone-seeking radionuclides which emit primarily -radiation, such as radium and uranium, pose a risk in the development of bone sarcoma (Mays et al. 1985). Osteosarcoma has been induced in mammals exposed to isotopes of U with a high specific activity (i.e. ^{232}U and ^{233}U) (Finkel 1953) and to enriched U (Filippova et al. 1978). No information on the effects of radiation in fish scales could be found.

Alpha radiation that is emitted by bone surface seekers (e.g. plutonium) is more effective in inducing osteosarcoma than that emitted by bone volume seekers (e.g. radium). Surface seekers emit alpha particles within the range of endosteal bone (tissue within 10 μm of bone surfaces), the critical site for the induction of bone cancer (ICRP 1977). As U is both a surface and a volume seeker, its ability to produce osteosarcoma is expected to lie intermediately between radium and plutonium (Stevens et al. 1980).

Cumulative doses to bone that have caused bone cancer in laboratory animals range from 56 rads from ^{239}Pu to 275 rads from ^{226}Ra. Thus, it is postulated that the critical radiation dose from U deposited in mammalian bone that is associated with a risk of developing bone sarcoma would lie in the range of 56 to 275 rads (Stevens et al. 1980). In the case of ^{226}Ra, no significant increases in bone cancer occur at doses to bone of less than 10 rads and only negligible risks at doses less than 100 rads (Cohen 1980).

Conversely, humans injected with Thorotrast, a thorium-containing substance used as a radiographic contrast medium, exhibited a higher incidence of bone cancer than reference populations. The estimated dose rates to bone in Thorotrast patients who died from osteosarcoma ranged from only 6 to 125 mGy/yr (approximately 0.6 to 12.5 rads/yr) (Hunacek and Kathren 1995).

The maximum estimated annual dose to lake whitefish bone is 2.96 ± 0.24 rads/yr, occurring in fish fed 10 000 μg U/g food for 100 days. This maximum dose rate to bone is far below the estimated critical doses reported in experimental mammals (Cohen 1980, Stevens et al. 1980). Although the dose rate of 2.96± 0.24 rads/yr is within the range reported for Thorotrast patients (Hunacek and Kathren 1995), it is unlikely to pose a significant carcinogenic risk in the skeletal tissues of freshwater fish.

C. Environmental Relevance

The dosimetric calculations employed in this study demonstrate that radiation doses incurred in lake whitefish within the exposure period were negligible. Thus, these results support the assumption that the adverse effects observed in lake whitefish fed diets contaminated with U arose via chemical pathways. Furthermore, these estimates illustrate potential doses that may arise in feral fish exposed to U under similar exposure regimes and potential hazards associated with prolonged exposure.

A number of factors render the dosimetric model employed here conservative. By disregarding ^{235}U, and assuming all accumulated U is ^{238}U, the calculated doses underestimate actual doses as the former isotope has a much greater activity than the

latter. Furthermore, feral fish are also exposed to ^{234}U, also a more potent radio-emitter than ^{238}U.

Dosimetric models applied to teleosts are conservative, relative to those applied to mammals, because radiation weighting factors are not employed. The lack of discrimination between the biological effectiveness of different forms of radiation in fish may tend to underestimate the risks associated with internally deposited U in lake whitefish. Thus, the use of data obtained in fish exposed to external radiation for evaluating the relevance of dose rates in the tissues of lake whitefish is limited and possibly invalid.

The radiation doses calculated here further underestimate the actual doses incurred in lake whitefish in this study because radiation emitted from food and faeces present in the gastrointestinal system was not considered. Furthermore, this model discounts other sources of radiation that are present in the natural environment. Feral fish residing in an aquatic system contaminated by U would also be exposed to external gamma emissions from U contained in the water, sediments, and in other biota. Lastly, U accumulated in feral fish would emit higher doses of radiation than in the present case because natural U contains a greater proportion of ^{235}U and ^{234}U, isotopes with much higher activities than ^{238}U, than the uranyl acetate used here.

Alpha radiation doses from internally deposited uranium were negligible within the length of the exposure period, with the possible exception of intestine in the highest treatment group. Potential radiation hazards may be of significant concern in intestines, kidney, and gonads over more prolonged exposures, in the order of years. Further

research into the risks associated with low-level internal radiation is required to appropriately evaluate these concerns.

Appendix 1.

Morphological, physiological, biochemical, and histological morphometric

parameters of the lake whitefish (*Coregonus clupeaformis*).

Abstract

In light of the current mining boom in northern Canada, there has been a call for research on fish species residing in these habitats (Lemly 1994). Although the lake whitefish is an abundant and ubiquitously distributed species in the Canadian environment, data regarding the basic physiological, biochemical, and histological parameters of this species are scarce. Because this teleost is a benthic feeder, it is at risk to contaminants which accumulate in sediments and is, therefore, an environmentally relevant surrogate for toxicological evaluations. This manuscript is a synthesis of parameters of laboratory-reared lake whitefish that were unaffected by U exposure in the study presented in chapters two and three. Data presented include: morphological and haematological parameters, MT concentrations in liver and kidney, and morphometric measurements of hepatocytes in liver and the second segment of proximal tubules in posterior kidney. Potential sex- and morphometric-related influences on MT concentrations were also evaluated. As MT is a widely used biomarker of metal exposure in feral fish (e.g. Olsson 1996), these analyses contribute to our understanding of endogenous factors known to influence MT concentrations in fish tissues. These data are to serve as contributions to published databases of freshwater fish which aid in the design and interpretation of laboratory and field evaluations of contaminant-induced effects in these biota.

I. Introduction

In light of the current mining boom in northern Canada, there has been a call for basic biological research on fish species which reside in these habitats, in order to reliably assess the potential hazards of these activities (Lemly 1994). Among those species for which additional research has been advocated, is the lake whitefish (*Coregonus clupeaformis*). Lake whitefish are ubiquitously distributed in the Canadian freshwater environment and are of considerable economic and commercial importance in Canada. As this species is a common resident of northern habitats where mining and milling activities are prevalent, it is among those species of fish at risk to contaminant enrichment of aquatic systems. Because the lake whitefish is a benthic feeding species (Scott and Crossman 1973), it is particularly susceptible to trophic transfer of contaminants, which accumulate in sediments.

For the above reasons, the lake whitefish is an environmentally relevant species for study of the accumulation and toxicology of contaminants occurring in aquatic ecosystems. However, few researchers have selected this species as a surrogate for toxicological evaluations and thus, there is a paucity of baseline data on physiological and biochemical characteristics of this species.

This manuscript is a synthesis of physiological, histological, and biochemical data on the lake whitefish that is to serve as a contribution to published databases of fish physiology and biochemistry. Data presented here are an assembly of parameters of laboratory-reared lake whitefish that were found to be unaffected by dietary U exposure (chapters two and three). These parameters include: fish weight, length, growth, condition

factor (CF), liver somatic index (LSI), haematocrit, serum osmolality, serum K^+, Cl^-, and

Na^+, liver and kidney metallothionein (MT), and histological morphometric parameters of

liver and posterior kidney. Parameters were also evaluated for sex- and morphometric-

related trends, in order to ascertain their usefulness as contaminant biomarkers and due to

concomitant gonadal maturation which occurred in the lake whitefish used in this study.

II. Materials and Methods

Data regarding fish, tank chemistry, fish holding, fish sampling, and tissue

sampling are described in chapter two. Methods used in the processing of histological

samples, slide preparation, and analyses of haematological variables, histological

morphometrics, and metallothionein concentrations are provided in chapter three.

Histological Morphometrics

Morphometric parameters in the second segment of the proximal tubule (P2) from

the posterior kidney of lake whitefish were directly measured by projecting microscopic

images onto a digitizer (Summagraphics Bit Pad, Fairfield, CT) using the SigmaScan

(version 3.90) software package (Jandel Scientific, Corte Madera, CA). For each fish,

measurements of tubular epithelium cell height (TEH), lumen diameter (LD), and tubular

diameter (TD) were made on the first 15 cross sections of P2's that were encountered

while moving from the top to the bottom of the centre-most section of posterior kidney on

a slide. TEH was measured from the basement membrane to the tip of the luminal brush

border, at four sites lying 90° from each other, and averaged for each tubule. LD was

obtained by measuring the distances between opposing tips of the lumina brush borders. Two distances were taken along planes lying 90° from each other and averaged. TD's were obtained from the average of the widest and shortest distances across the tubules. Lumen to tubule diameter ratios, expressed as a percentage, were calculated: LD / TD * 100. This parameter serves as an indicator of tubular dimensions and may be used to demonstrate dilation or contraction of tubules or reductions in brush border heights.

Hepatocyte morphometrics were obtained using the same apparatus as used for kidney. For each fish, the diameters of nuclei were measured in the first 50 cells with spherical nuclei that were encountered while moving downward from the top of the centre-most liver tissue section on a slide. Relative hepatocyte size (i.e. area) was estimated by counting the number of hepatocyte nuclei in two fields of standard area (18 000 μm^2) of a liver section. Nuclear areas were calculated, from the formula πr^2. Cytoplasm areas were obtained by subtracting nuclear areas from estimates of hepatocyte size. Nuclear area to cytoplasmic area ratios (N:C) were then generated from the latter values.

Statistical Analysis

All data for parameters of lake whitefish that were not affected by U treatment in the study presented in chapters two and three are presented here as mean ± SE. Data have been separated by sampling day and by sex to evaluate potential influences of gonadal maturation and sex upon the various parameters. Data were analyzed using the SigmaStat 2.0 Software Package (Jandel Scientific, Corte Madera, CA). Sex differences were

evaluated using a Student's t-test within each sampling day and within all data combined. Linear regression analysis was applied to evaluate the influence of fish morphometrics on the concentration of MT in liver of lake whitefish. The latter evaluations were conducted on each sex individually for data obtained on days 10, 30, 100. The influence of fish morphometrics on morphometric parameters of hepatocytes (sexes and sampling days pooled) was also evaluated by linear regression analysis. A significance level of $\alpha = 0.05$ was used for all evaluations, unless otherwise indicated.

III. Results

Reproductive Rhythm

The lake whitefish used in this study underwent gonadal maturation during the course of the evaluation. Of the three sampling periods, the ovaries of fish were in the most advanced state of maturation on sampling day 30. By day 100, eggs were being resorbed, however, in some individuals the ovaries had begun to redevelop.

Ovarian growth was not anticipated in this study as the environmental variables, including photoperiod, water temperature, pH, and dissolved oxygen, known to affect and potentially stimulate sexual development in fish, were held constant. Other chemical variables of the incoming water did not vary significantly during the exposure period. Because of the potential for reproductive physiology to influence the variables

investigated here, data are presented for each sex on each sampling day, for parameters where differences between the sexes were found.

Morphometrics

Lake whitefish weight, length, CF, growth, and LSI according to sex and sampling time are presented in Table App. 1.1. No significant sex-related differences were found for any parameter within each sampling day or within all data pooled.

Metallothionein

Concentrations of MT in liver and kidney of males, females, and of both sexes pooled are presented in Table App. 1.2. Significantly higher concentrations of MT were observed in the livers of females relative to males on sampling day 30 and when data from all sampling days were pooled. There were no significant differences between MT concentrations in the kidney of males and females.

There were no significant correlations between fish length, weight, liver weight, LSI, or CF and hepatic MT concentrations in males or females on days 10 or 30 or in males sampled on day 100. There were significant positive correlations between hepatic MT and weight ($P < 0.05$), liver weight ($P < 0.05$), and CF ($P < 0.100$) of females sampled on day 100 (Figure App. 1.1).

Haematological Variables

The haematological variables, serum K^+, Na^+, and Cl^-, haematocrit, serum osmolality, and $Na^+ + Cl^-$/osmolality, of lake whitefish as a function of sex and sampling time are presented in Table App. 1.3. Serum Na^+, Cl^-, haematocrit, and $Na^+ + Cl^-$ / osmolality were significantly lower in females than males sampled on day 10. When data from all sampling times were pooled, the only parameter which was significantly different between the sexes was $Na^+ + Cl^-$ / osmolality.

Histological Morphometrics in Posterior Kidney and Liver

The morphometric parameters of the second segment of the proximal tubule in the posterior kidney of lake whitefish are presented for females, males, and both sexes pooled in Table App. 1.4. No significant sex-related differences were observed.

Mean lake whitefish relative hepatocyte areas, hepatocyte nuclear diameters, and N:C are presented in Table App. 1.5. Because no sex-related differences were found, data from both sexes (nine females and nine males) were pooled and analyzed by linear regression for the influence of fish morphometrics. Significant correlations and the level of significance are presented in Table App. 1.5 and illustrated in Figure App. 1.2. All parameters were significantly correlated to fish weight, most notably, the N:C ratio. The relative areas of hepatocytes decreased with increasing fish weights, whereas the diameters of hepatocyte nuclei and the N:C ratios were positively correlated to fish weights. The latter parameter was also positively correlated· to fish length and liver weight.

IV. Discussion

Reproductive Rhythm

The most likely explanation for gonadal maturation in lake whitefish used in this study was the persistence of an endogenous rhythm. Fish may maintain circannual reproductive rhythms despite constant laboratory conditions (Sumpter 1990) and it has been suggested that there is sufficient evidence for an endogenous obligatory reproductive cycle in salmonids (Scott 1990, Sumpter 1990). This situation may also apply to lake whitefish in the present study. The timing of gonadal development in the experimental lake whitefish corresponded to spawning times of natural populations. The experiment was conducted over winter and feral lake whitefish in Canada spawn in the fall or early winter (Scott and Crossman 1973).

Haematology

The concentrations of K^+, Na^+, and Cl^- in serum, serum osmolality, and the fractional contribution of Na^+ and Cl^- to serum osmolality ($Na^+ + Cl^-$ / osmolality) were similar to reported values for this species (Scherer et al. 1986) and within the range reported for salmonids (Folmar 1993). In a recent review of the literature on haematological parameters of teleosts, Folmar (1993) did not report the range of haematocrits typical of lake whitefish or other salmonids. However, the mean haematocrits observed in lake whitefish from this study were similar to values reported

for feral lake whitefish inhabiting two reference lakes included in a survey of the Beaverlodge Lake area, northern Saskatchewan (Bernstein and Swanson 1989).

Metallothionein

Metallothionein is an ubiquitous, low-molecular-weight (7 kDa), heat stable, cysteine-rich, heat shock protein, whose structure is remarkably similar across taxa (Kille et al. 1992). It has been identified in a number of fish tissues including liver, anterior and posterior kidney, spleen, pancreas, brain, ovaries, testes, heart, gills, and intestines (Chan et al. 1988, Chatterjee and Maiti 1987, Hao et al. 1993, Hylland et al. 1994). Under non-pathological physiological conditions, the primary function of this protein is believed to be its participation in the maintenance of copper and zinc homeostasis (Olsson 1996). MT synthesis is induced by trace amounts of non-essential metals Cd, Hg, and Ag and excessive levels of Cu and Zn in fish (Roesijadi 1992, Wood et al. 1996). In turn, MT sequesters these metals, thus functioning as what is most often interpreted as a detoxification protein (Roesijadi 1992).

MT induction in tissues of feral fish inhabiting metal-contaminated environments has received a great deal of attention and is advocated as a reliable biomarker of metal exposure (Deniseger et al. 1990, Hamilton and Mehrle 1986, Hogstrand et al. 1989, Klaverkamp et al. 1991, Olsson and Haux 1986, Petering et al. 1990). Thus, it is of use to compile base-line MT data for lake whitefish, an abundant species in the Canadian environment.

Hepatic concentrations of MT in this study are approximately twice those found in feral lake whitefish from lakes in northern Saskatchewan impacted by U mining and milling (Klaverkamp et al. 1997). This suggests that all fish from the present study may have been experiencing MT induction possibly attributable to reproductive physiology (Olsson 1996). Comparisons to other species suggest that lake whitefish kidneys contain similar concentrations as burbot (*Lota lota*) and rainbow trout (*Oncorhynchus mykiss*) and a much lower concentration than a number of other freshwater species. Reported mean ± SE renal MT concentrations for other species include: 131 ± 13 in longnose suckers (*Catostomus catostomus*), 79.3 ± 8.1 in northern pike (*Esox lucius*), 69.9 ± 6.6 in flathead chub (*Platygobio gracilis*), 18.9 ± 3.0 in burbot (Klaverkamp et al. 1996), and 7.64 ± 0.45 in rainbow trout (Roch et al. 1982).

The significantly higher concentrations of hepatic MT in female lake whitefish, relative to males, on sampling day 30 could be a result of gonadal maturation, which was at the most advanced state at this time. Similar results have been found in feral fish during the reproductive period. Female plaice (*Pleuronectes platessa* L.) contained elevated concentrations of hepatic MT during the period of gonadal maturation, relative to non-spawning females or males, presumably a result of egg development (Overnell et al. 1987). Large increases, from 160 μg/g to 580 μg/g, in the concentrations of MT in livers of laboratory-reared female rainbow trout (*Salmo gairdneri*) were also noted during the spawning season (Olsson et al. 1987). Generally, MT and Zn concentrations in liver fluctuate throughout the annual reproductive cycle of rainbow trout (Olsson et al. 1987).

Liver MT and vitellogenin production peak during the early stages of egg development, when the ovaries are approximately 20% of their maximal size (Overnell et al. 1987).

The significant positive correlations that were observed between liver MT and lake whitefish morphometric variables have not been reported in the literature. However, numerous other variables have been demonstrated to influence the occurrence and extent of MT induction in fish such as reproductive state (Olsson and Kling 1995, Olsson et al. 1987, Overnell et al. 1988), sex (Olsson et al. 1987, Overnell et al. 1988, Schlenk et al. 1996), species (Cope et al. 1994), age (Cope et al. 1994), temperature (Hyllner et al. 1989) and route of metal exposure (Overnell et al. 1988). Furthermore, MT concentrations may vary considerably between individuals of the same species and population (Cosson et al. 1991, Overnell et al. 1987).

Histological Morphometrics

Histopathology is a useful tool in the diagnosis of contaminant exposure and toxicity in feral fish. This technique is advantageous because it provides immediate information regarding fish health and permits evaluation of integrated responses. Furthermore, histological analyses facilitate examination of specific structures and cells within a heterogenous tissue; a differentiation which can not be made in bioassays of tissue homogenates.

Alterations in the sizes of cells, structures, and organelles may indicate sub-lethal or lethal cytotoxicity (Hinton and Lauren 1990a, b, Hinton et al. 1992) and examination of hepatocyte morphometrics has received a great deal of attention by aquatic

toxicologists (Hinton and Lauren 1990a, b, Hinton et al. 1988, Hinton et al. 1992). However, to the author's knowledge, there are no published data on hepatocyte morphometrics of this species of fish. Furthermore, in a 1990 review of histological biomarkers in fish, Hinton and Lauren (1990b) reported that no kidney morphometric data for any fish species could be found in the literature. The author is unaware of any publications since this review.

The use of kidney histopathologies as biomarkers of contaminant exposure are currently considered promising candidates for application in the future with the advent of additional supporting research (Hinton et al. 1992). Renal lesions are not yet established biomarkers due to the inadequate volume of histopathological research on this tissue and the lack of corroborating results of field and laboratory studies. In order to develop histological biomarkers that could be reliably applied to monitoring of feral fish populations inhabiting impacted systems, it is necessary to assemble base-line data for a variety of fish species. The morphometrics reported here serve as contributions to this database.

V. Conclusions

This manuscript provides a synthesis of measured morphological, haematological, biochemical, and histological morphometric parameters in laboratory-reared lake whitefish. The immediate purpose of this undertaking is to amass base-line data for this species, in the published literature. Ultimately, it is hoped that with further contributions of this nature, the lake whitefish may be more frequently selected as a laboratory

surrogate for the evaluation of aquatic contaminants. The advantages of this undertaking include its benthic habit, its ubiquitous distribution in the Canadian environment, its commercial and recreational importance, and the basic need to obtain toxicological information on a greater number of teleost species.

Table App. 1.1. Lake whitefish morphometrics, wet weight, fork length, CF, growth, growth rate, and LSI. Data are expressed as mean (± SE, n) of females, males, and both sexes pooled.

Day	Sex	Wet Weight (g)	Fork Length (cm)	CF (%)	Growth (% increase in vvw)	Growth Rate (g/fish/d)	LSI (%)
10	Females	675 (41, 14)	35.4 (0.7, 14)	1.50 (0.03, 14)	4.97 (0.63, 14)	1.16 (0.13, 14)	0.792 (0.021, 14)
	Males	668 (24, 10)	37.7 (1.3, 10)	1.28 (0.07, 10)	4.53 (0.62, 10)	1.07 (0.14, 10)	0.776 (0.049, 10)
	Both Sexes	672 (25, 24)	36.3 (0.7, 24)	1.41 (0.04, 24)	4.79 (0.44, 24)	1.12 (0.10, 24)	0.785 (0.023, 24)
30	Females	702 (20, 15)	35.8 (0.4, 15)	1.53 (0.02, 15)	9.18 (1.68, 15)	1.15 (0.22, 15)	0.783 (0.029, 15)
	Males	642 (67, 8)	34.7 (1.2, 8)	1.50 (0.05, 8)	11.3 (2.3, 8)	1.29 (0.31, 8)	0.761 (0.046, 8)
	Both Sexes	681 (26, 23)	35.4 (0.5, 23)	1.52 (0.02, 23)	9.92 (1.34, 23)	1.20 (0.18, 23)	0.775 (0.024, 23)
100	Females	712 (65, 9)	36.3 (1.0, 9)	1.45 (0.05, 9)	17.7 (2.6, 9)	1.00 (0.13, 9)	0.907 (0.067, 9)
	Males	771 (33, 15)	37.1 (0.4, 15)	1.51 (0.04, 15)	16.9 (1.5, 15)	1.09 (0.09, 15)	0.784 (0.047, 11)
	Both Sexes	749 (32, 24)	36.8 (0.5, 24)	1.49 (0.03, 24)	17.2 (1.3, 24)	1.06 (0.07, 24)	0.831 (0.040, 18)
All Days	Females	694 (22, 38)	35.8 (0.4, 38)	1.50 (0.02, 38)	9.6 (1.2, 38)	1.12 (0.10, 38)	0.810 (0.020, 36)
	Males	709 (25, 33)	36.7 (0.5, 33)	1.44 (0.04, 33)	11.8 (1.3, 33)	1.13 (0.09, 33)	0.775 (0.027, 29)
	Both Sexes	701 (16, 71)	36.2 (0.3, 71)	1.47 (0.02, 71)	10.6 (0.9, 71)	1.13 (0.07, 71)	0.795 (0.016, 65)

Table App. 1.2. Metallothionein concentrations, expressed as mean (± SE, n), in liver

and kidney of lake whitefish. Significant sex-related differences are indicated by their

respective P-values. NS = not significantly different at P > 0.05.

Day	Liver MT (µg/g ww)				Kidney MT (µg/g ww)			
	Both sexes	Females (F)	Males (M)	F > M	Both sexes	Females (F)	Males (M)	F > M
10	346.9 (42.8, 18)	390.9 (66.8, 10)	291.9 (45.3, 8)	NS	12.3 (0.9, 24)	11.8 (1.0, 24)	12.9 (1.6, 10)	NS
30	350.4 (29.7, 23)	412.9 (32.8, 15)	233.4 (30.3, 8)	P < 0.005	14.1 (1.1, 23)	15.3 (1.5, 15)	11.8 (1.1, 8)	NS
100	380.8 (46.5, 24)	379.9 (97.1, 9)	381.3 (49.5, 15)	NS	19.3 (1.5, 18)	19.8 (4.1, 6)	19.0 (1.2, 12)	NS[†]
All	360.7 (23.1, 65)	397.7 (34.2, 34)	320.1 (29.4, 31)	P < 0.100	14.8 (0.7, 65)	14.7 (1.1, 35)	15.0 (1.0, 30)	NS[†]

[†] Mann-Whitney Rank Sum Test

Table App. 1.3. Lake whitefish haematological variables serum K^+, Na^+, Cl^-, haematocrit, serum osmolality, and $Na^+ + Cl^-$ / osmolality. Data are expressed as mean ($\pm$ SE, n) of females, males, and both sexes pooled. Significant sex-related differences are indicated by their respective P-value. NS = not significantly different at $P > 0.05$.

(A)

Day	Serum K+ (mM/L)				Serum Na+ (mM/L)				Serum Cl- (meq/L)			
	Both Sexes	Females (F)	Males (M)	F ≠ M	Both Sexes	Females (F)	Males (M)	F ≠ M	Both Sexes	Females (F)	Males (M)	F ≠ M
10	2.76 (0.10, 24)	2.84 (0.14, 14)	2.65 (0.15, 10)	NS	152.9 (1.0, 24)	151.6 (1.6, 14)	154.9 (0.4, 10)	P < 0.05†	131.7 (1.7, 24)	129.6 (2.8, 14)	134.6 (0.7, 10)	P < 0.05†
30	3.03 (0.11, 23)	2.96 (0.11, 15)	3.18 (0.25, 8)	NS	155.0 (0.36, 23)	154.9 (0.4, 15)	155.3 (0.7, 8)	NS	130.6 (0.5, 12)	130.9 (0.7, 8)	130.0 (0.6, 4)	NS
100	2.92 (0.10, 24)	2.83 (0.14, 9)	2.98 (0.13, 15)	NS	151.7 (0.4, 24)	151.7 (0.6, 9)	151.7 (0.6, 15)	NS	134.0 (0.6, 24)	134.6 (0.6, 9)	133.7 (0.9, 15)	NS
All	2.90 (0.06, 71)	2.88 (0.07, 38)	2.93 (1.0, 33)	NS	153.2 (0.4, 71)	152.9 (0.7, 38)	153.5 (0.4, 33)	NS	132.4 (0.7, 60)	131.4 (1.3, 31)	133.5 (0.6, 29)	NS

(B)

Day	Haematocrit (%)				Serum Osmolality (mOsm/L)				Na$^+$ + Cl$^-$ / Osmolality (%)			
	Both Sexes	Females (F)	Males (M)	F ≠ M	Both Sexes	Females (F)	Males (M)	F ≠ M	Both Sexes	Females (F)	Males (M)	F ≠ M
10	41.3 (0.8, 24)	40.0 (0.9, 14)	43.1 (1.2, 10)	P < 0.05[†]	316.5 (2.0, 24)	315.0 (3.3, 14)	318.7 (1.3, 10)	NS	89.9 (0.4, 24)	89.2 (0.6, 14)	90.8 (0.6, 10)	P < 0.05
30	41.4 (1.1, 23)	40.6 (1.4, 15)	42.9 (1.6, 8)	NS	321.2 (1.6, 18)	322.2 (2.0, 13)	318.6 (2.7, 5)	NS	89.2 (0.4, 23)	89.0 (0.5, 15)	89.6 (0.6, 8)	NS
100	36.8 (1.5, 24)	38.7 (0.9, 9)	35.6 (2.3, 15)	NS	314.5 (0.9, 24)	314.4 (0.7, 9)	314.5 (1.4, 15)	NS	90.9 (0.3, 24)	91.1 (0.4, 9)	90.8 (0.4, 15)	NS
All	39.8 (0.7, 71)	39.9 (0.7, 38)	39.6 (1.3, 33)	NS	317.1 (1.0, 66)	317.4 (1.6, 36)	316.6 (1.0, 30)	NS	90.0 (0.2, 71)	89.5 (0.3, 38)	90.5 (0.3, 33)	P < 0.05

† Mann-Whitney Rank Sum Test

Table App. 1.4. Lake whitefish proximal tubule (P2) morphometrics: epithelial cell heights, lumen diameters, tubule diameters, and lumen diameter : tubule diameter ratios. Data are expressed as mean (± SE, n) of males, females, and both sexes pooled.

	Epithelium Cell Height (μm)	Lumen Diameter (μm)	Tubule Diameter (μm)	Lumen Diameter : Tubule Diameter (μm)
Both Sexes	17.4 (0.1, 71)	19.8 (0.3, 71)	54.6 (0.5, 71)	0.362 (0.025, 71)
Females	17.3 (0.2, 38)	19.6 (0.4, 38)	54.1 (0.8, 38)	0.360 (0.004, 38)
Males	17.5 (0.2, 33)	20.1 (0.5, 33)	55.1 (0.7, 33)	0.364 (0.026, 33)

Table App. 1.5. Lake whitefish hepatocyte morphometrics, relative hepatocyte area, nuclear diameter, and nucleus area : cytoplasmic area ratio (N:C) and correlations to fish morphometrics, length, weight, liver weight, LSI, and CF. Significant correlations are indicated with their respective P-value. NS = no significant correlation (P > 0.100).

	n	Relative Hepatocyte Area (μm^2)	Mean Nuclear Diameter (μm)	N:C Ratio (%)
Mean (± SE)	18	162 (6)	5.44 (0.05)	17.2 (0.7)
Fork Length (cm)	18	NS	NS	P = 0.012
Wet Weight (g)	18	P = 0.088	P = 0.081	P = 0.003
Liver Weight (g)	18	NS	NS	P = 0.018
LSI (%)	18	NS	NS	NS
CF (%)	18	NS	NS	NS

Figure App. 1.1. Hepatic MT in female lake whitefish sampled on day 100 as a function of morphometric parameters. Linear regressions of (A) Log MT vs. log wet weight, (B) Log MT vs. log liver weight, and (C) MT vs. CF. $P < 0.05$.

(A)
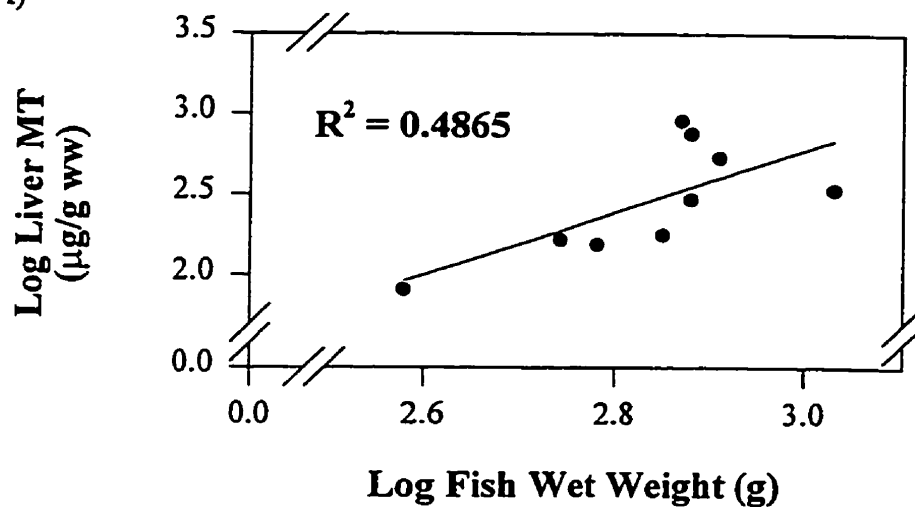
(B)
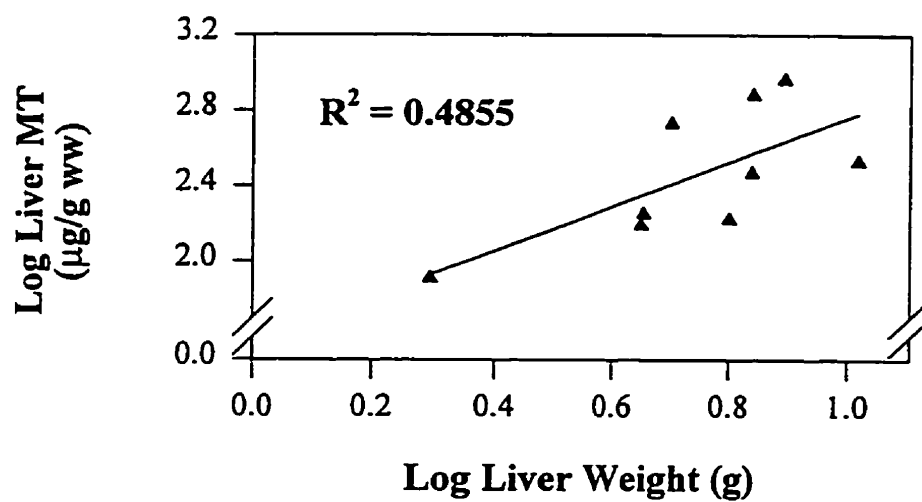
(C)
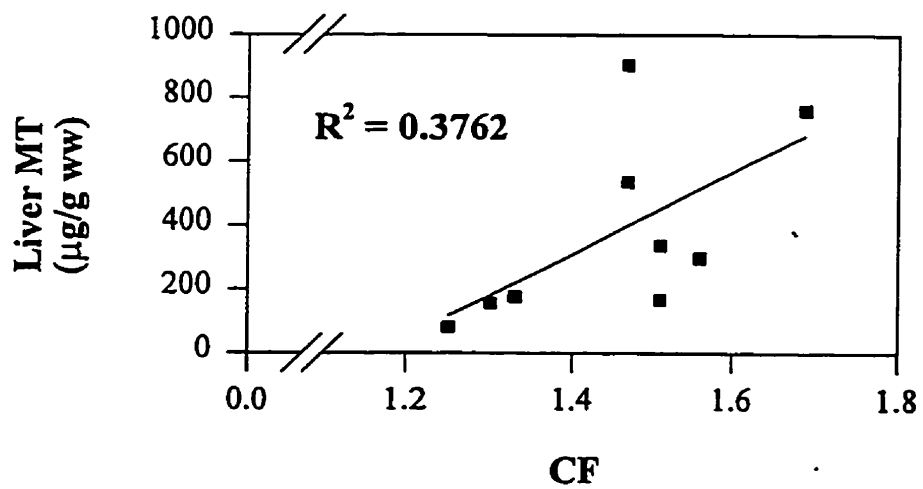
Figure App. 1.2. Lake whitefish hepatocyte morphometrics, relative hepatocyte area, nuclear diameter, and nuclear area to cytoplasmic area ratio (N:C), as a function of fish morphometrics. Linear regressions of (A) Relative hepatocyte area vs. fish weight, (B) Nuclear diameter vs. fish weight, (C) Hepatocyte N:C vs. fish weight, (D) Hepatocyte N:C vs. fish length, and (E) Hepatocyte N:C vs. liver weight.

(A)

(B)

(C)
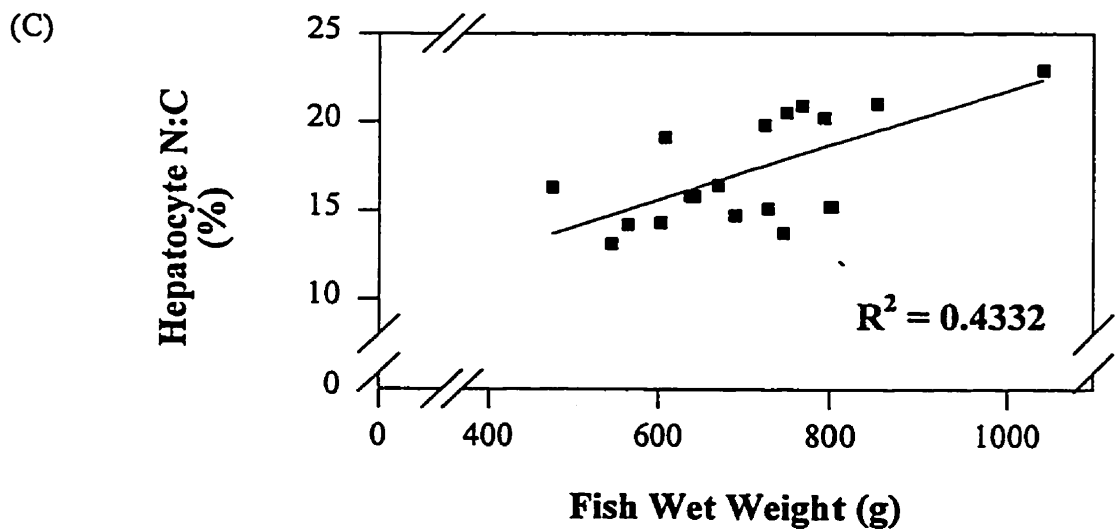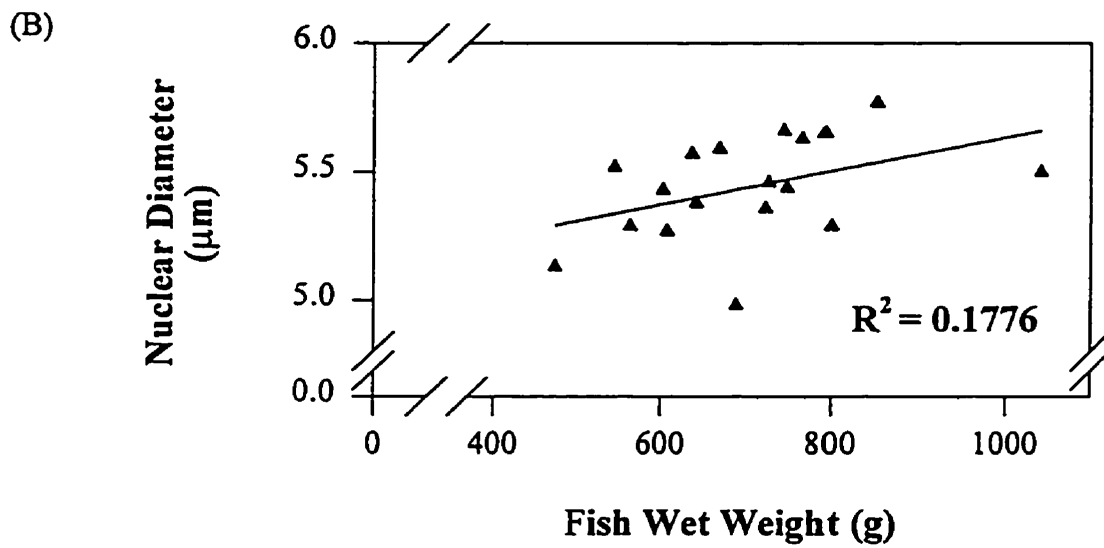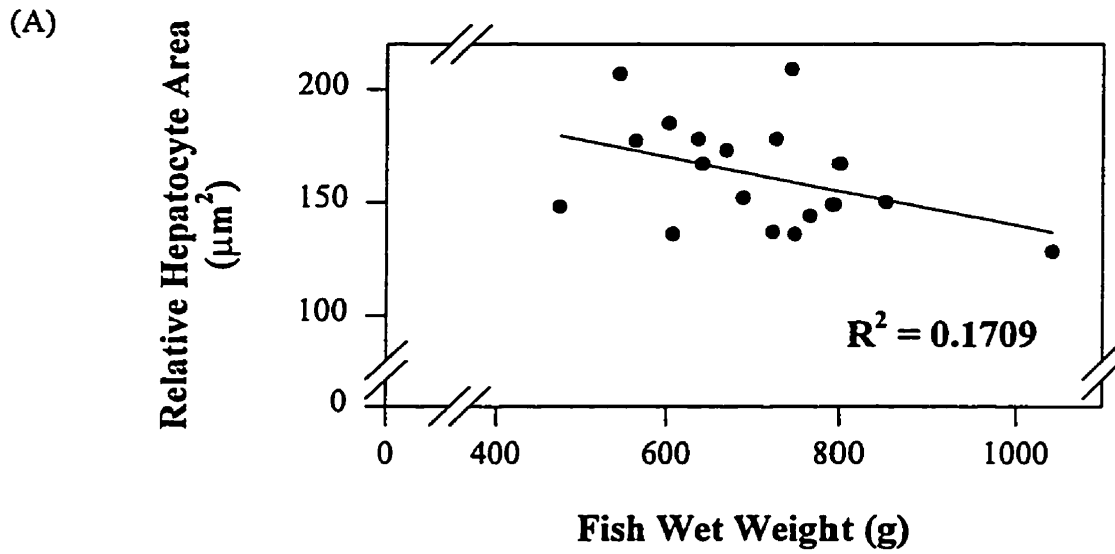
(D)
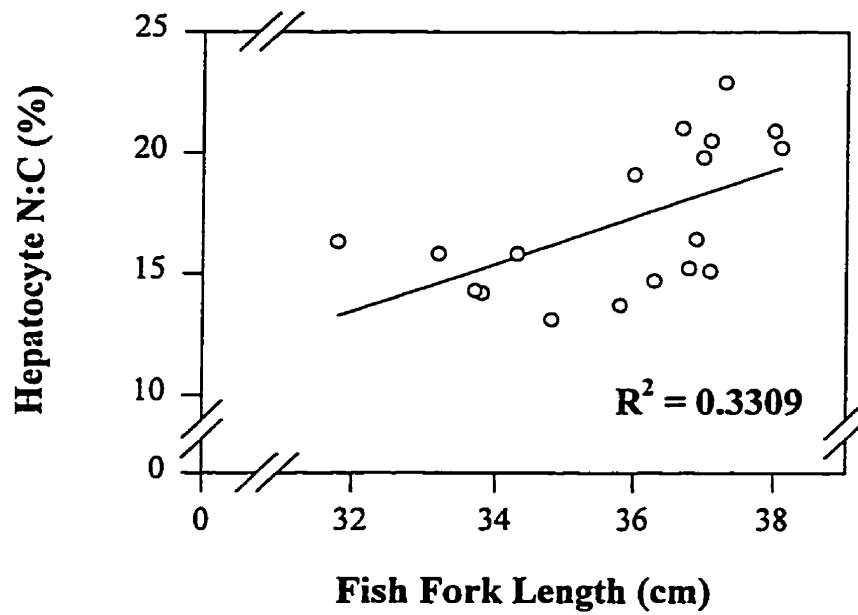
(E)
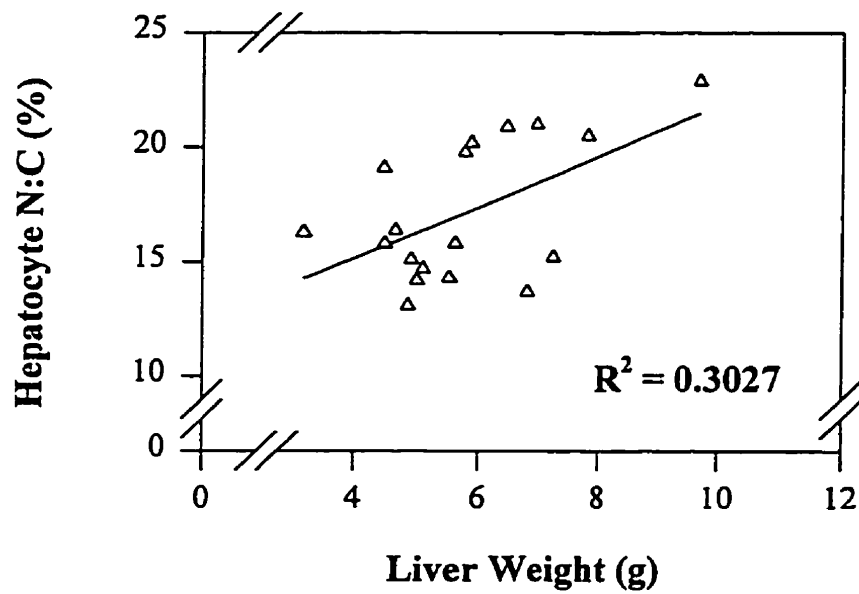
Appendix 2.

Table App. 2.1. Uranium in the Canadian aquatic environment: Sediment concentrations. The assembled data include concentrations of U in the sediments and surface waters of aquatic ecosystems impacted by U mining and milling and from other sources of U pollution. The pollution status of each system is classified as background (no source of pollution), low (natural enrichment, site distant from source of pollution), and polluted (receives direct inputs of U and sediments and/or water contain above background [U]). Data are expressed as mean (± SE), unless otherwise indicated. Not reported (NR), standard deviation (SD).

Location	Pollution Status	[U] Sediments μg/g (± SE) dw	Sediment Depth cm	[U] Surface Water μg/L (± SE)	Reference
Canada: Background					
Streams and lakes across Canada	Pristine	Mean: 5.6 Range: 1.45-24.7			NRCC (1983)
Saskatchewan					
Beaverlodge Lake					
Beaverlodge Lake System	Polluted			180-3400 (1979)	Swanson (1983)
Fulton Lake, Reference site	Background - Low			3.7 - 7.6	Swanson (1983)
Beaverlodge Lake System	Polluted	Beaverlodge Lake: 109.22 (21.87) Tailings System: 12.18 - 85.55 All ww	Ekman Grab	Beaverlodge Lake: 338 (193) Tailings System: 3700 - 4300	Swanson (1985)
Fredette & Milliken Lakes (Reference systems)	Background - Low	3.22 & 6.60	Ekman Grab	1.20 & 3.25	Swanson (1985)

Location	Pollution Status	[U] Sediments μg/g (± SE) dw	Sediment Depth cm	[U] Surface Water μg/L (± SE)	Reference
Cluff Lake					
Island Lake, (Cluff Lake mine & mill)	Polluted	440.9 (SD 303.1)	0 - 10 cm		Hynes et al. (1987)
		20.7 (SD 18.8)	10 - 20 cm		
Key Lake					
Wolf & Fox Lakes	Polluted	Peak: 180	5 cm		J.F. Klaverkamp (Freshwater Institute, 501 University Crescent, Winnipeg, Manitoba, Personal Comm.)
Little McDonald Lake	Polluted	Peak: 1100	0 - 2 cm		
Rabbit Lake					
Effluent Creek	Polluted	Peak: 575	0 - 2 cm		J.F. Klaverkamp (Freshwater Institute, 501 University Crescent, Winnipeg, Manitoba, Personal Comm.)
Park Creek	Unknown	< 50 μg/g	0 - 20 cm		

Location	Pollution Status	[U] Sediments µg/g (± SE) dw	Sediment Depth cm	[U] Surface Water µg/L (± SE)	Reference
Hidden Bay, Wollaston Lake	Polluted	326	0 - 2 cm		Neame et al. (1982)
Upper Link Lake, Wollaston Lake	Polluted	5650	0 - 2 cm		Neame et al. (1982)
Drilling Site, Wollaston Lake	Polluted	271	0 - 2 cm		Neame et al. (1982)
Collins Bay, Wollaston Lake	Reference	3.4	0 - 2 cm		Neame et al. (1982)
Lake Athabasca					
Langley Bay, Lake Athabasca	Polluted	< 10 - 3000	peaks generally at 3 - 4 cm		Joshi et al. (1989)
Langley Bay, Lake Athabasca	Polluted	12.5 - 52.0	Ekman (1 - 2 cm) + top 1 cm with free-fall corer + hand scooping 1 - 2 cm	2.3 & 3.0	Waite et al. (1989)

Location	Pollution Status	[U] Sediments μg/g (± SE) dw	Sediment Depth cm	[U] Surface Water μg/L (± SE)	Reference
Langley Bay, Lake Athabasca	Polluted	8.2 - 16.9	Ekman grab	0.2 - 280	Waite et al. (1988)
Langley Bay, Lake Athabasca	Polluted	1.2 - 7.1 Bq/g U-234 & U-238	Ekman grab	0.004 - 2.51 Bq/g U-234 & U-238	Platford and Joshi (1988)
Lake Athabasca, Saskatchewan	Reference	1.1	Ekman grab	0.2	Waite et al. (1988)
Uranium City					
Surface waters near Uranium City	Polluted			Mean: 3700 Max: 94000	Kalin (1988)
Ontario					
Quirke Lake, Serpent River Watershed	Polluted	> 1000	0 - 2 cm		McKee et al. (1987)
Holding pond and West Lake; West Arm of Elliot Lake tailings site	Polluted			170 - 370	Moffett and Tellier (1978)

Location	Pollution Status	[U] Sediments µg/g (± SE) dw	Sediment Depth cm	[U] Surface Water µg/L (± SE)	Reference
Surface waters near Elliot Lake tailings	Polluted			Mean: 60 Max: 10 100	Kalin (1988)
Surface waters near Bancroft Tailings	Polluted			Mean: 100 Max: 1100	Kalin (1988)
Surface waters near Elliot Lake and Bancroft Areas	Reference			Mean: 300 Max: 6000	Kalin (1988)
Northwest Territories					
Lakes & Creeks, Rayrock	Polluted			0.5 - 78	Veska and Eaton (1991)
British Columbia					
Okanagan Highlands	Naturally enriched	10.32	NR	0.34	Mahon (1982)

Location	Pollution Status	[U] Sediments μg/g (± SE) dw	Sediment Depth cm	[U] Surface Water μg/L (± SE)	Reference
Other Sources of U					
Ontario					
Port Hope Harbour, Lake Ontario	Polluted	120 - 18000	0 - 2 cm		Hart et al. (1986)
Port Hope Harbour, Lake Ontario	Polluted			> 20 000	Hart et al. (1986)
Port Hope Harbour, Lake Ontario	Polluted	Mean: 92.5 Range: 3.5 - 1280	Ekman grab	Mean: 92.5 Range: 3.5-1280	Hart et al. (1986)

Appendix 3.

Table App. 3.1. Uranium in the global environment: sediment concentrations. The assembled data include concentrations of U in the sediments and surface waters of global aquatic ecosystems impacted by U mining and milling and from other sources of U pollution. The pollution status of each system is classified as background (no source of pollution), naturally elevated (site naturally enriched in U), low (site distant from source of pollution, above background but low sediment and/or water [U]), and polluted (receives direct inputs of U and sediments and/or water contain above background [U]). Data are expressed as mean (± SE), unless otherwise indicated. Not reported (NR).

Location	Pollution Status	Source of U	[U] Sediments µg/g dw	Sediment Depth cm	[U] Surface Water µg/L	Reference
Bohemia						
River Mze (River Elbe)	Polluted	U mining and milling	approximately 250 µg/g (maximum)	Cori's sampler	30	Justyn and Stanek (1974)
Russia						
Lake Issyk-Kul', Russia	Naturally elevated	Natural enrichment: erosion of rocks	1.36 - 22	"Muds"	30	Koval'skiy and Vorotnitskaya (1965), Kovalsky et al. (1967)
Sweden						
Liljuthatten (central)	Polluted	Prospecting / drilling & excavating	3.2 - 554	NR		Pettersson et al. (1988)
Pleutajokk (north)	Polluted		3.9 - 12			Pettersson et al. (1988)

Location	Pollution Status	Source of U	[U] Sediments µg/g dw	Sediment Depth cm	[U] Surface Water µg/L	Reference
United States						
Midnite Mine Discharge, Spokane, Washington	Polluted	Tailings leachate			1820	Nichols and Scholz (1989)
Blue Creek, Spokane, Washington	Polluted	Tailings leachate		55 - 212		Nichols and Scholz (1989)
Indian Creek, Gunnison, Colorado	Low - moderately polluted	Periodic runoff from U mine			100 - 4000	Parkhurst et al. (1984)
Yugoslavia						
Rivers, Sora Region	Polluted	Mine wastes & processing plant	2.04 - 9.83	NR	0.11 - 12.5 mg/m³	Stegnar and Kobal (1982)

Location	Pollution Status	Source of U	[U] Sediments µg/g dry	Sediment Depth cm	[U] Surface Water µg/L	Reference
Other Sources of U						
United States						
U Pond, Hanford Site, Washington	Polluted	Various wastes	650	Upper 10 cm		Emery et al. (1981)
Tims Branch, U.S. Savannah	Polluted	Nuclear fuel production and target assemblies for reactors	6165			Keklak et al. (1994)

Appendix 4.

Table App. 4.2. The acute toxicity of aqueous uranium to cold-water and warm-water freshwater fish. Toxicity of U (mg/L) is expressed as an LC50, a lowest-observed-effect-concentration (LOEC), or a no-observed-effect-concentration (NOEC). The toxicity endpoint is death, unless otherwise indicated.

	Effect	Concentration mg/L	Reference

Cold-water Species

Brook trout	*Salvelinus fontinalis*		
Juveniles Soft water 32 mg/L CaCO₃	96-h LC50	5.5	Parkhurst et al. (1984)
Hard water 210 mg/L CaCO₃		23	
Simulated Indian Creek water 184 mg/L CaCO₃	48-h LC50	59	
Eggs Simulated Indian Creek water 201 mg/L CaCO₃	Hatching Success Fry survival	no effect up to 9.08 no effect up to 9.08	
soft water 30 mg/L CaCO₃	96-h LC50	7.8	Parkurst et al. (1984)

Rainbow trout	*Salmo gairdneri*		
soft water 30 mg/L CaCO₃	96-h LC50	6.2	Parkurst et al. (1984)

Fathead Minnow	*Pimephales promelas*		
soft water 20 mg/L CaCO₃	96-hr LC50	2.8	Parkhurst et al. (1984)
hard water 400 mg/L CaCO₃		135	

	Effect	Concentration mg/L	Reference
soft water 20 mg/L CaCO₃	96-hr LC50		Tarzwell and Henderson (1960)
uranyl nitrate		3.1	
uranyl acetate		3.7	
juveniles 66-73 mg/L CaCO₃	96-hr LC50	16.7	Poston et al. (1984)
Colorado squawfish	*Ptychocheilus lucius*		
Larva	96-h LC50	46.0	Hamilton (1995)
Juvenile		46.0	
Bonytail	*Gila elegans*		
Larva	96-h LC50	46.0	Hamilton (1995)
Juvenile		46.0	
Razorback sucker	*Xyrauchen texanus*		
Larva	96-h LC50	46.0	Hamilton (1995)
Juvenile		46.0	
Zebra fish	*Brachidanio rerio*		
	24-h LC50	6.4	Vinot and Larpent (1984)
Flannelmouth Sucker	*Catostomus latipinnis*		
12 - 13 day-old larvae 144 mg/L CaCO₃	24 - 96 hr LC50	43.5	Hamilton and Buhl (1997)

	Effect	Concentration mg/L	Reference
Warm-water Species			
Black-banded rainbowfish	*Melanotaenia nigrans*		
7 and 90 days old	96-h LC50	1.70	Bywater et al. (1991)
Chequered rainbowfish	*Melanotaenia splendida inornata*		
7 days old	96-h LC50	2.66	Bywater et al.
90 days old		3.46	(1991)
Mariana's hardyhead	*Craterocephalus marianae*		
juvenile	96-h LC50	1.22	Bywater et al. (1991)
Delicate blue-eye	*Pseudomugil tenellus*		
juvenile	96-h LC50	0.73	Bywater et al. (1991)
Reticulated perchlet	*Ambassis macleayi*		
juvenile	96-h LC50	0.80	Bywater et al. (1991)
Purple-Spotted gudgeon	*Mogurnda mogurnda*		

	Effect	Concentration mg/L	Reference
7 days old	96-h LC50	1.11	Bywater et al. (1991)
90 days old		1.46	
Larva	LOEC reduced fish length	0.400	Holdway (1992)
	NOEC	0.200	

Appendix 5.

Formulae, sample calculations, and results of calculations of α-radiation doses of internally deposited uranium in the tissues of lake whitefish.

Internal α-radiation dose

The annual alpha radiation dose of internally deposited uranium was estimated for each tissue based upon U concentrations measured in the tissues at each sampling time. The most common formula, and that adopted here, employed for calculating α-radiation doses incurred from internally deposited radionuclides in fish is that developed by Woodhead (1974), as described in Joshi (1984):

$$D = 1.87 \times 10^{2-} \times E \times C_f$$

Where: $D = \alpha$- or ß-radiation dose in mrad/yr

E = Average Energy of Particle in MeV/disintegration

$C_f = [\text{radionuclide}]_{\text{fish}}$ in pCi/kg (Joshi, 1984)

Another similar and equivalent expression was derived by Harley (1974) for calculating dose rate to bone (in mammals) resulting from internally deposited radionuclides (obtained secondarily in Wrenn et al., 1985):

$$D = 18.7 \times E \times C_f$$

Where: D= dose rate in mrad/yr

C_f = radionuclide concentration in pCi/g

The radioisotopic content of uranyl acetate dihydrate (Fluka Chemical Corporation, Ronkonkoma, NY) is different than that of natural U, or U ore. Uranyl acetate (Fluka Chemical Corporation, Ronkonkoma, NY) is depleted of ^{235}U, and thus its specific activity is approximately one half that estimated by Veska and Eaton (1991) for natural U. Natural U is usually reported to have a total specific activity of 25 kBq/g U, whereas U contained in uranyl acetate (Fluka Chemical Corporation, Ronkonkoma, NY) has a reported specific activity of 0.187 μCi/g, equivalent to only 12.33 kBq/g U or 0.33291 pCi/g U. Differences between the isotopic ratios contained in uranyl acetate (Fluka Chemical Corporation, Ronkonkoma, NY) relative to natural U result in reduction in specific activity.

Where:	Uranyl Acetate	Natural U
^{238}U =	99.8%	99.2739%
^{235}U =	0.3%	0.7205%
^{234}U =	0.0%	0.0056%
	(Fluka Chemical Corp.)	(Veska and Eaton 1991)

The conversion factor of 1 g U = 0.33291 pCi generated from the above information was used in the conversion of U concentrations from chemical (i.e. μg U/g tissue) to radiological units (pCi U/g tissue) for the purposes of dosimetric calculations.

The average α-energy of U decay for the three isotopes comprising uranyl acetate and natural U are: ^{238}U 4.19 MeV/disintegration; ^{235}U 4.40 MeV/disintegration; and ^{234}U 4.76 MeV/disintegration (Mays et al. 1985). As the overwhelming fraction of U used in the present study is ^{238}U, E was accepted to be 4.19 MeV/disintegration for the purposes of dose calculations. The presence of ^{235}U was ignored.

Sample Dose Calculation For U in Bone (high treatment group d 100):

U Concentration in Bone (μg/g ww)	U Concentration in Bone (pCi/g ww)	α-Radiation Dose (mrad/yr)
114	38	2963

(1) Conversion to radiological units:

114 μg/g (ww) x 0.33291 pCi/μg U = 38 pCi/g tissue (ww)

(2) Dose calculation:

D = 18.7 x 4.19 MeV x 38 pCi/g (ww)

= 2963 mrads/yr

Assumptions

1. U is distributed evenly throughout each tissue

2. Tissues are of a uniform shape

3. U is at equilibrium in the tissues

4. In the case of bone, that U accreted in underlying bone layers will behave as that on the surface of the tissue

5. Radiation is emitted in all directions, uniformly

6. Surface losses are negligible

7. No major redistributions or excretions of U will occur

Table App. 5.1. Calculated alpha radiation doses of internally deposited U in nine tissues

of lake whitefish fed contaminated diets for (A) 10, (B) 30, and (C) 100 days. Data are

expressed as mean (± SE).

(A) Day 10

Treatment	Dose (mrads/yr)									
(μg U/g)	Bone	Gill	Gonad	Intestine	Kidney	Liver	Muscle	Scales	Skin	Total
0	82 (0)	22 (0)	22 (3)	47 (0)	41 (0)	27 (0)	16 (0)	19 (0)	16 (0)	292
100	82 (0)	22 (0)	27 (3)	47 (0)	41 (0)	27 (0)	16 (0)	19 (0)	16 (0)	297
1000	82 (0)	22 (0)	28 (6)	171 (50)	41 (0)	27 (0)	16 (0)	85 (19)	16 (0)	488
10 000	302 (59)	38 (16)	184 (113)	12 500 (5870)	137 (38)	43 (10)	33 (13)	300 (17)	35 (10)	13 500

(B) Day 30

Treatment (μg U/g)	Dose (mrads/yr)									
	Bone	Gill	Gonad	Intestine	Kidney	Liver	Muscle	Scales	Skin	Total
0	82 (0)	22 (0)	24 (4)	47 (0)	41 (0)	27 (0)	16 (0)	19 (0)	16 (0)	294
100	82 (0)	22 (0)	19 (3)	47 (0)	41 (0)	27 (0)	16 (0)	19 (0)	21 (5)	294
1000	98 (17)	39 (17)	68 (32)	241 (85)	63 (14)	33 (6)	16 (0)	127 (22)	33 (6)	718
10 000	479 (54)	368 (198)	3220 (1330)	16 400 (936)	149 (36)	68 (7)	23 (6)	831 (99)	66 (27)	21 600

(C) Day 100

Treatment (μg U/g)	Dose (mrads/yr)									
	Bone	Gill	Gonad	Intestine	Kidney	Liver	Muscle	Scales	Skin	Total
0	82 (0)	22 (0)	27 (0)	47 (0)	41 (0)	27 (0)	16 (0)	19 (0)	16 (0)	297
100	82 (0)	22 (0)	27 (3)	47 (0)	41 (0)	27 (0)	16 (0)	97 (7)	16 (0)	375
1000	273 (51)	22 (0)	35 (12)	365 (61)	141 (40)	42 (15)	19 (3)	239 (39)	30 (7)	1170
10 000	2960 (235)	77 (17)	1230 (517)	22 900 (9620)	430 (77)	284 (64)	21 (4)	1950 (226)	49 (12)	29 900

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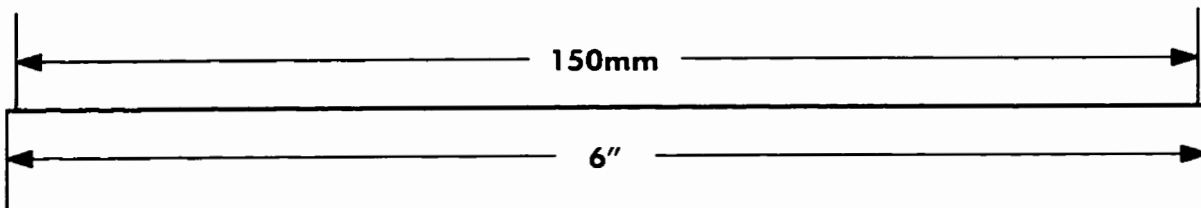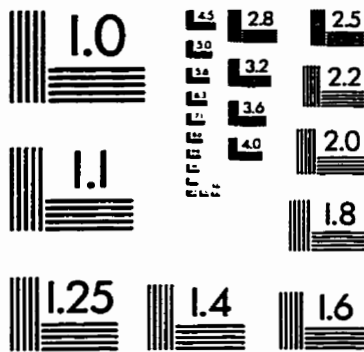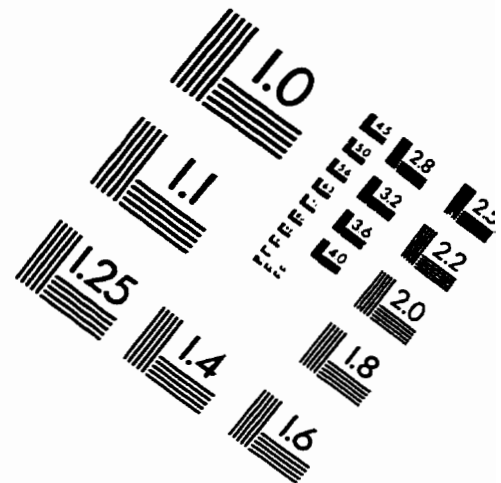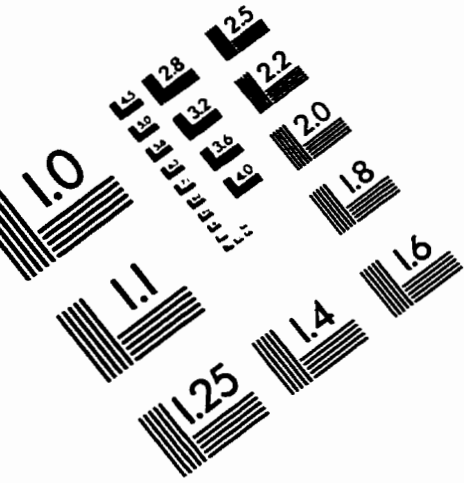
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IMAGE EVALUATION
TEST TARGET (QA-3)