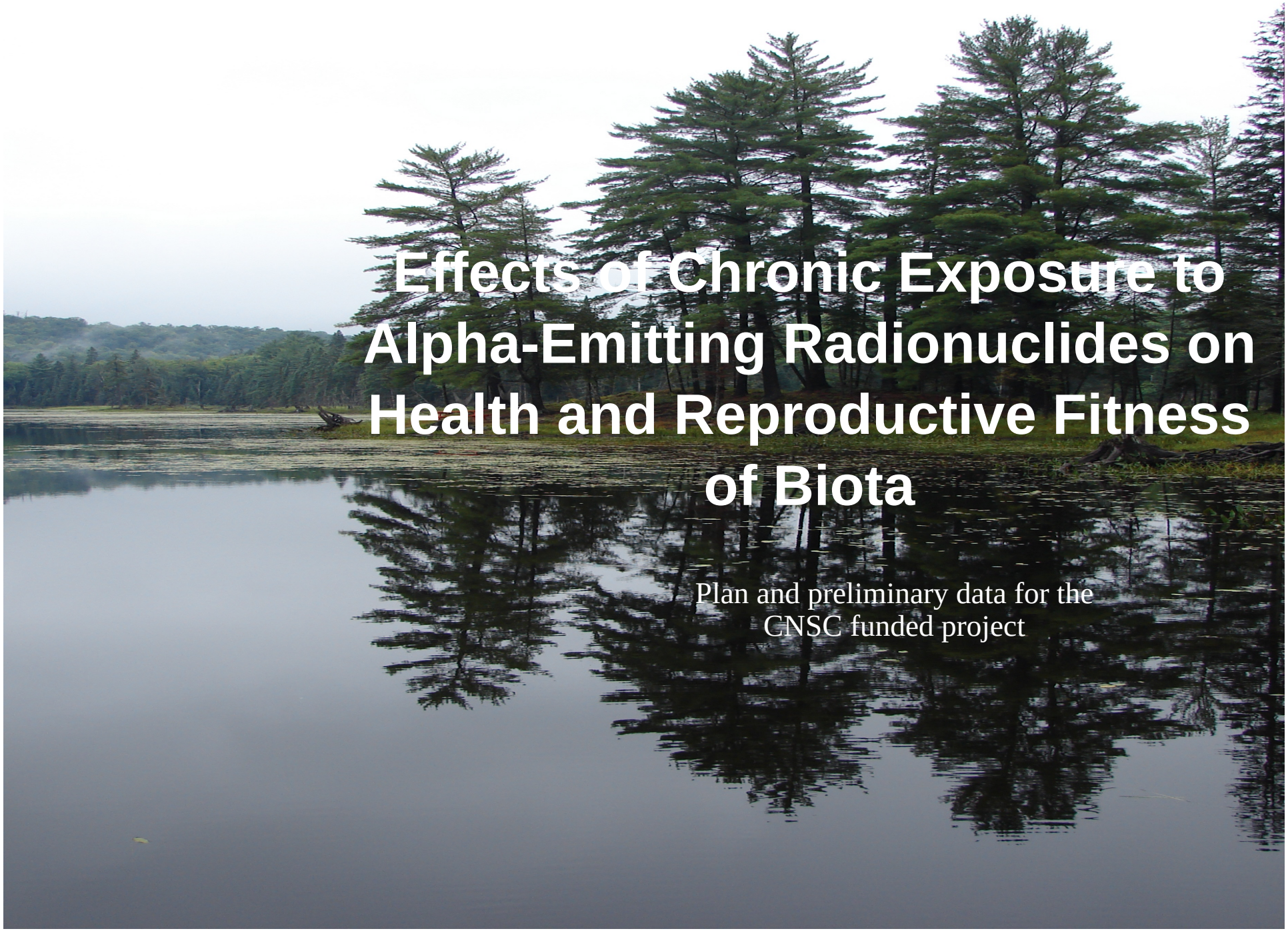
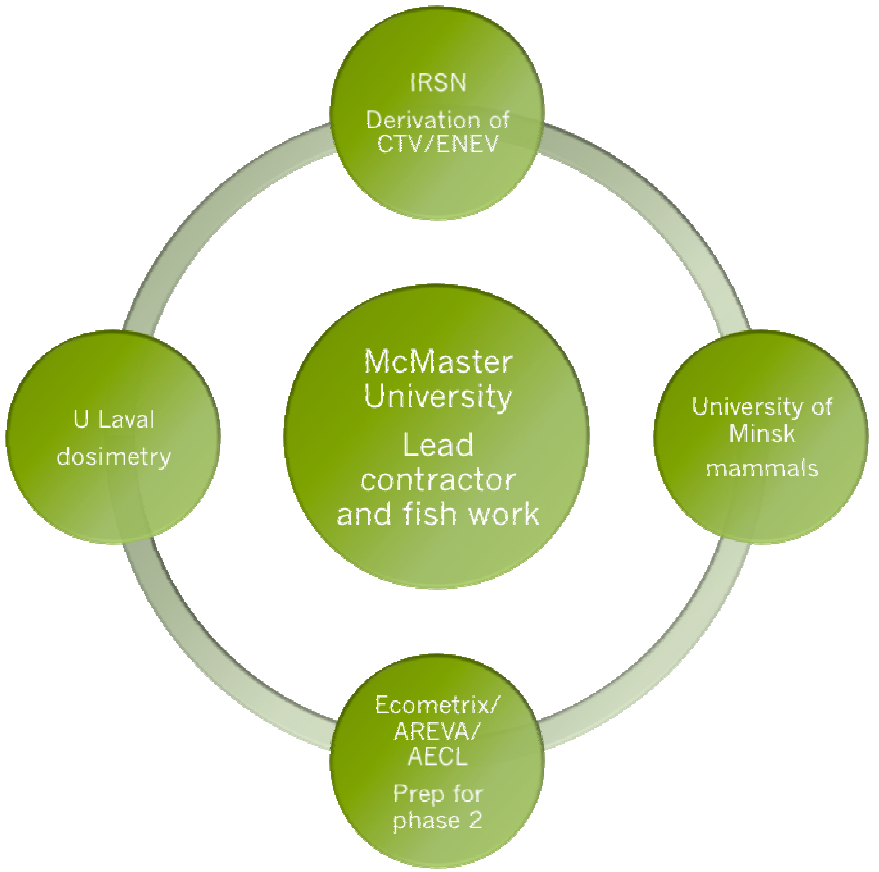


Effects of Chronic Exposure to Alpha-Emitting Radionuclides on Health and Reproductive Fitness of Biota

Plan and preliminary data for the
GNSC funded project

Management structure and partner responsibilities



Study the multigenerational reproductive and health effects of chronic lifetime exposure of a fish model (fathead minnow) to ingestion of alpha-emitting radionuclides (e.g. Ra-226, Po-210)

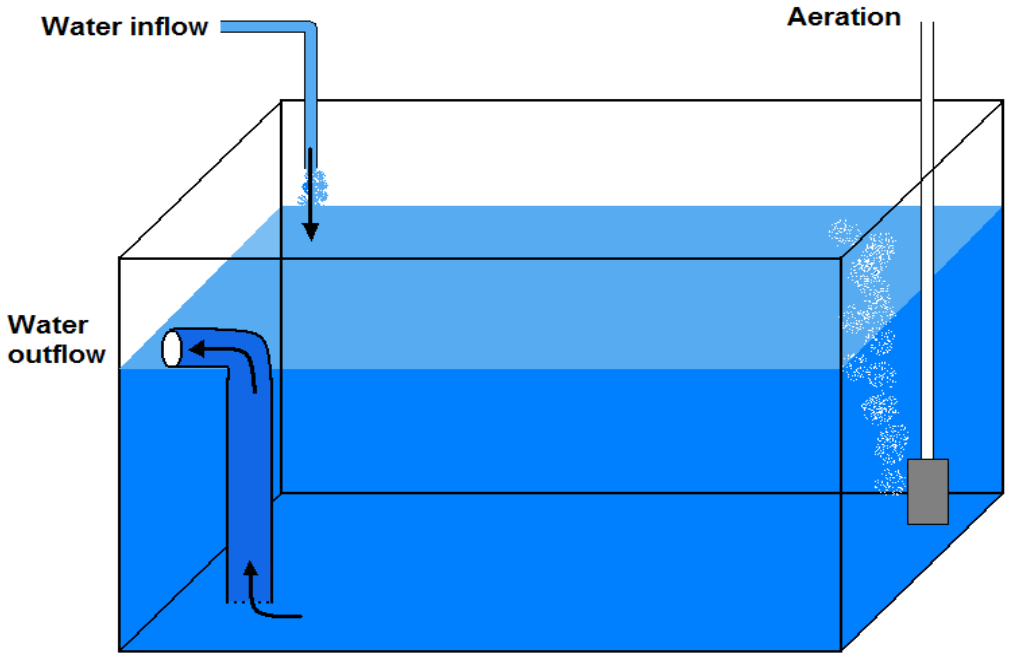
McMaster University

Fathead minnow

Life span – 2 years if spawned, 4 years if not.

Sexual maturity reached in 1 year. Eggs deposited under low ledge (1 cm height)

Fathead minnow husbandry



Water temperature: 12 - 15^{o}C

Standing water volume: 25 l

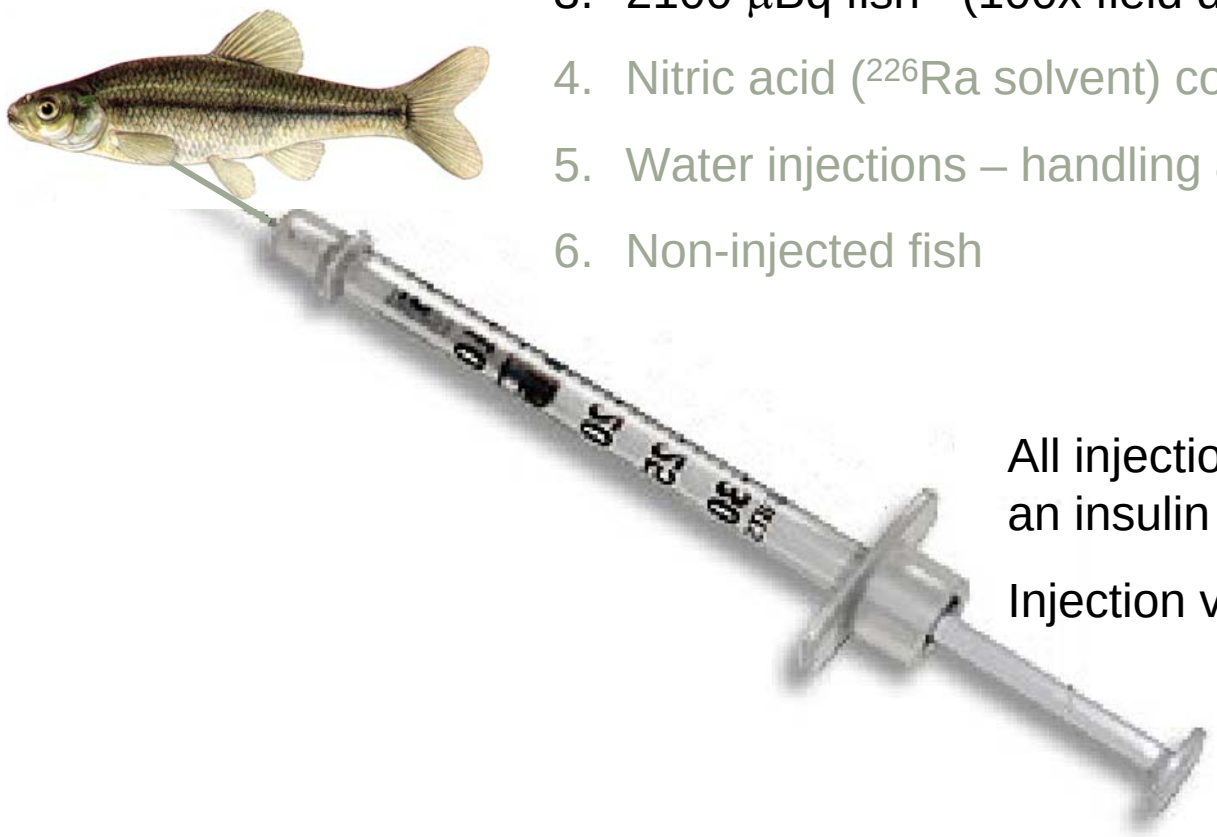
Water flow through: 250 ml min^{-1}

Feeding: once daily, to satiation

NOTE: for breeding water temp increased to 23^{o}C and ledge provided

Preliminary acute injection experiment: Fathead minnow ^{226}Ra injections

1. 21 μBq fish^{-1} (dose based on fathead minnow field data; Clulow et al 1998)
2. 210 μBq fish^{-1} (10x field data dose)
3. 2100 μBq fish^{-1} (100x field data dose)
4. Nitric acid (^{226}Ra solvent) control injections
5. Water injections – handling & injection stress control
6. Non-injected fish

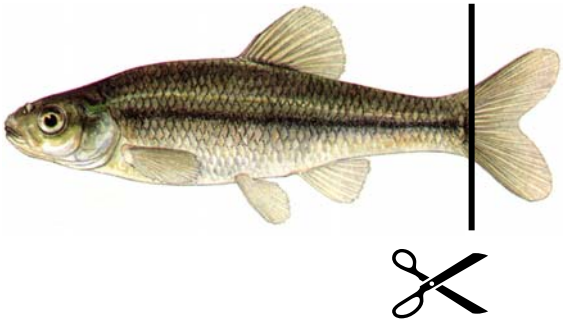


All injections administered i.p. via an insulin syringe (29G needle)

Injection volume = 3μl fish^{-1}

Clulow et al, 1998. Env. Pol. 99: 13 – 28

Fathead minnow post-injection analysis



24h.

Caudal fin samples taken for analysis of apoptosis and stress signal.

Gills collected for proteomic analysis

Whole body collected for dosimetry

^{226}Ra injected fish Non-injected fish

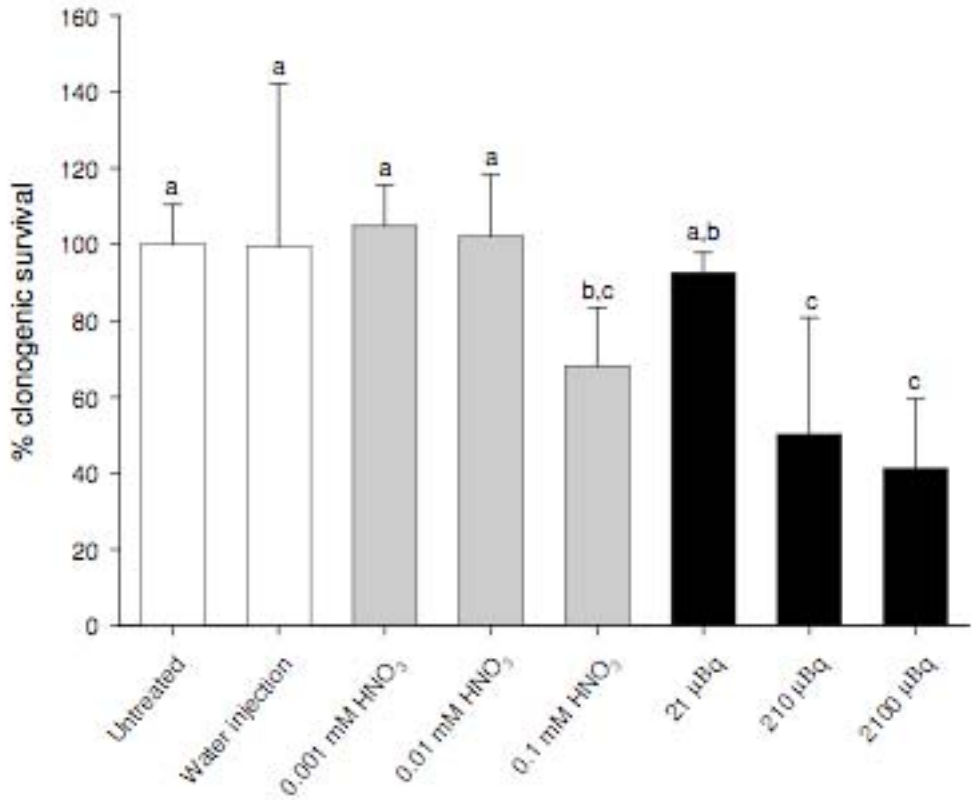
Late time point (TBD)

Evaluation of ^{226}Ra induced bystander effect on non-injected fathead minnow

Gills collected for proteomic analysis

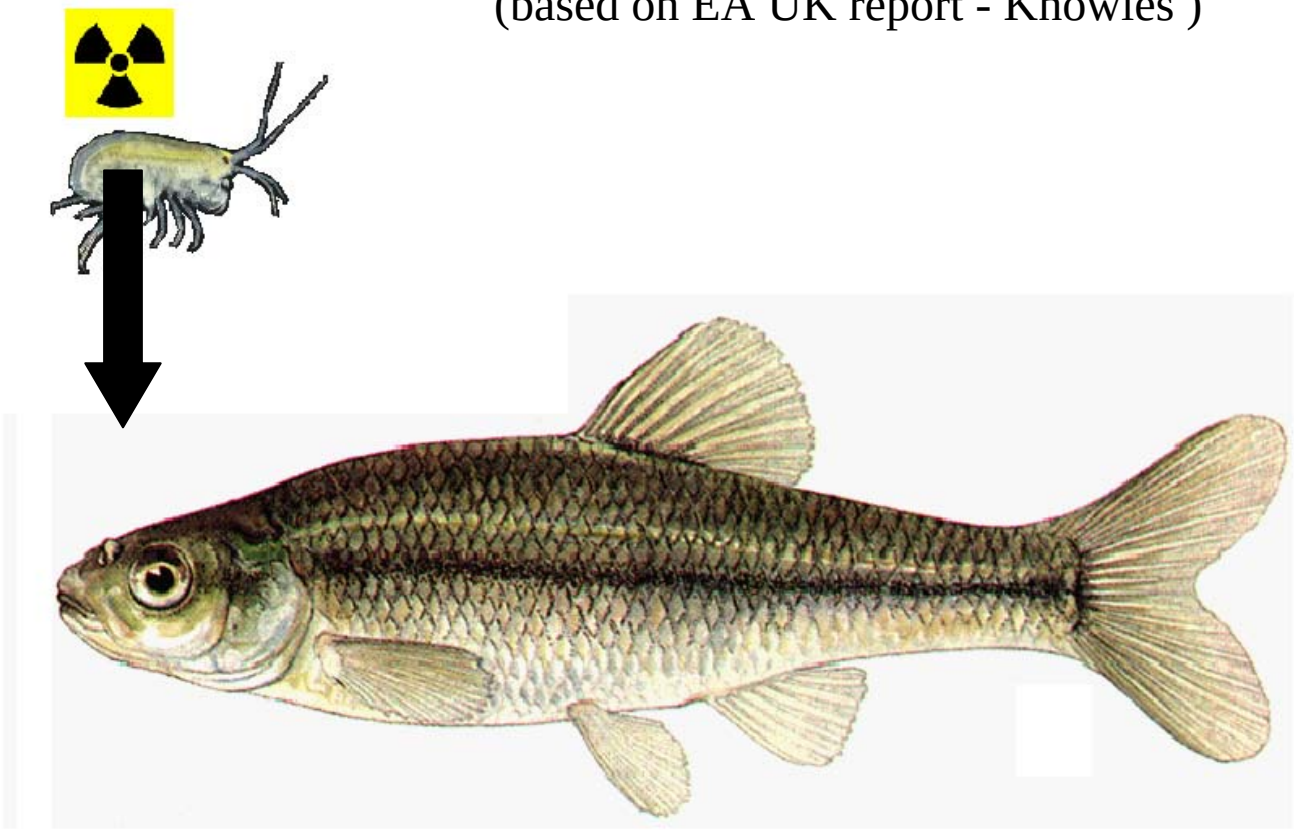
Whole body dosimetry

Stress signal assay result

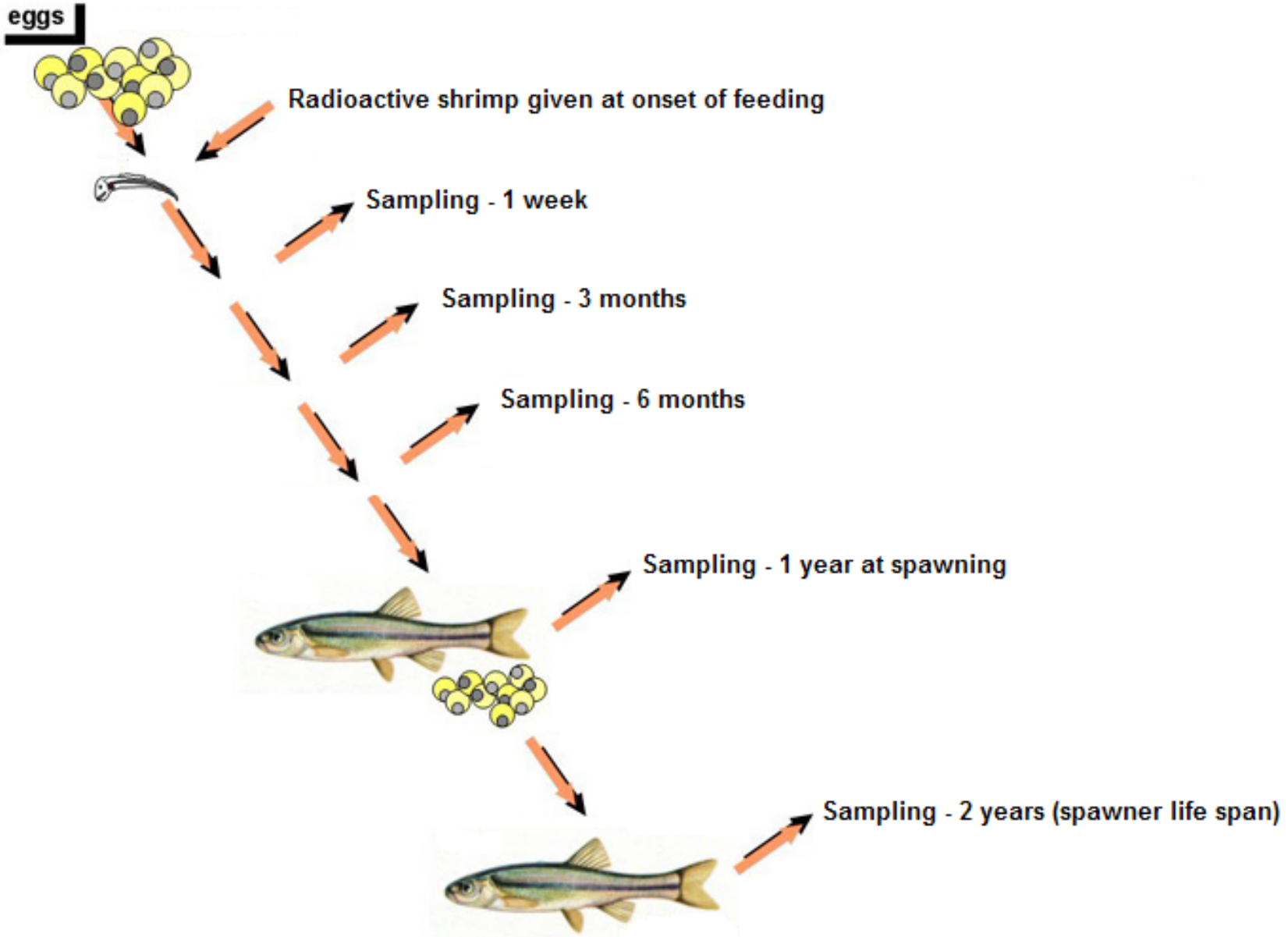


Ingestion approach

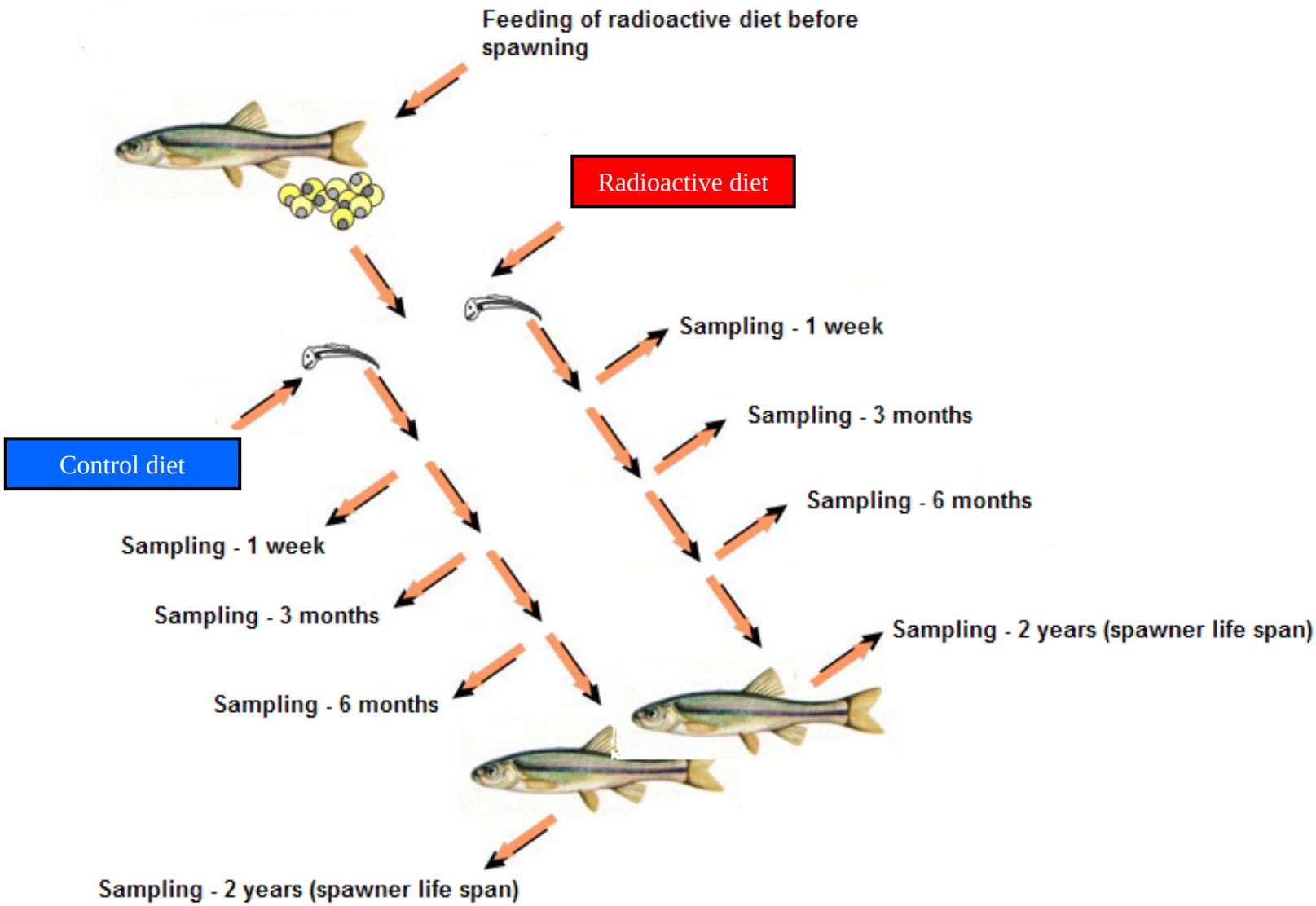
Max dose will be in the region of 400microGray/day
(based on EA UK report - Knowles)



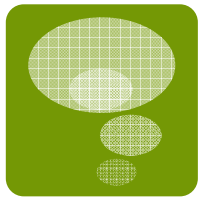
Chronic exposure Experimental outline



Experimental outline 2

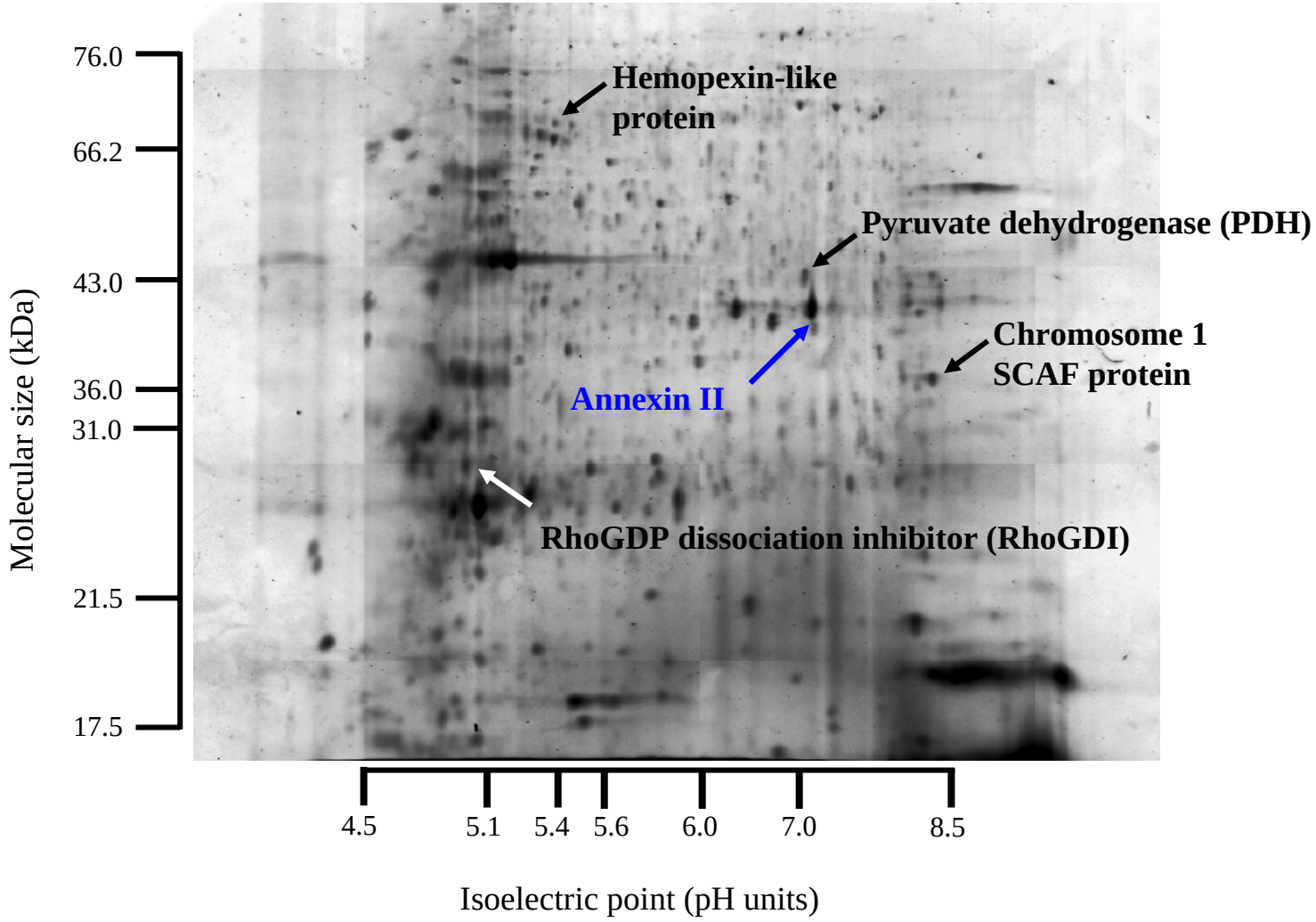


Endpoint summary



Parameter	Physiology / biomarker
Biochemical growth indices (RNA, DNA, protein).	Depending on tissue analyses – index of growth process (hyperplasia / hypertrophy), potential reproductive fitness, potential change in metabolic activity.
Physical growth indices	Weight and physical parameters
Fecundity/fertility	Egg production and viability of offspring
Macrophage superoxide production.	Non-specific immunity.
Proteomics.	Precise molecular changes to the suite of proteins synthesised.
Apolipoprotein A1 expression.	Ability to metabolise cholesterol and, in the gill, maintenance of epithelial barrier function.

Proteomics. 2D gels separates proteins according to isoelectric point and molecular size.

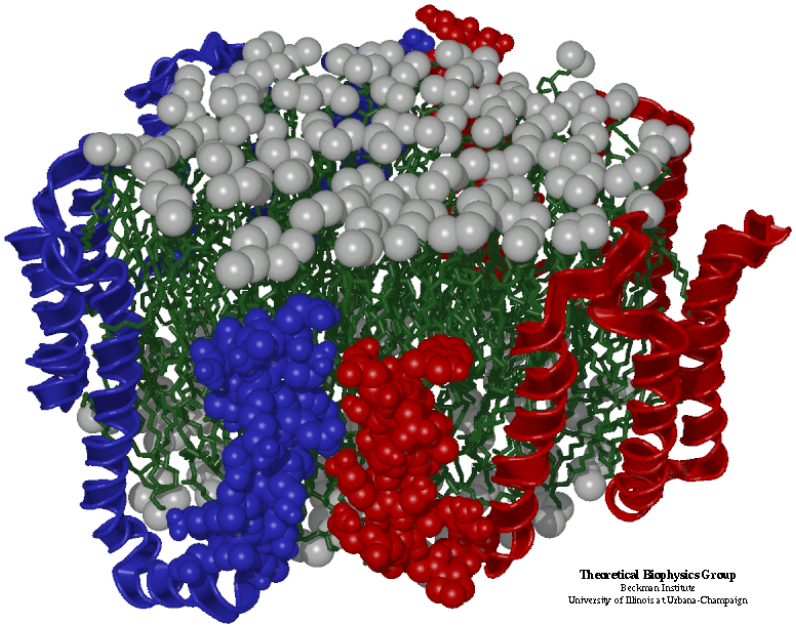


Rainbow trout gill proteome indicating proteins affected by radiation and bystander effect

APOLIPOPROTEIN AI

In fish - instrumental in the regeneration of fin (Monnot et al, 1999) and nerve (Harel et al, 1990)

- Protective / restorative role observed in fish tissues



Apolipoprotein A1 regulates cholesterol transport.

Synthesised in liver

Lead reduces cholesterol in brain, testes and ovary, and increases cholestrol in liver, in catfish (*Clarius batrachus*).

(Katti and Sathyanesan,1983).

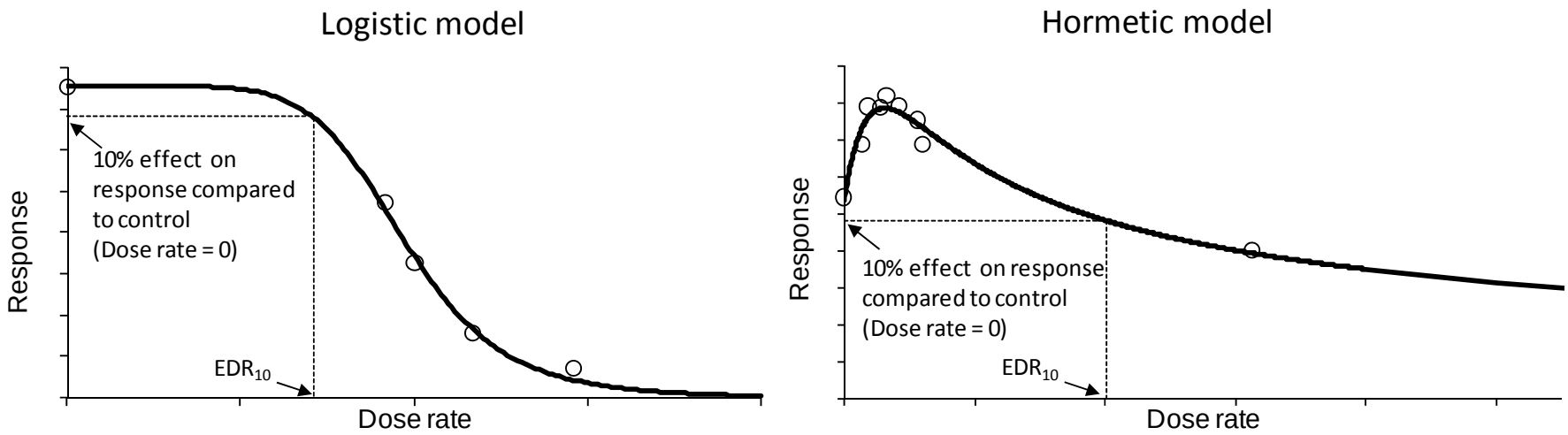
Apolipoprotein A1 measured by ELISA and elevated in gamma irradiated fish

Apolipoprotein A1 expression; a biomarker of Po exposure?

Based on the results, derive Critical Toxicity Values (CTVs) and Expected No Effects Values (ENEVs) for fish and mammals in terms of daily intake rates, equilibrium tissue concentrations, and dose to critical tissues

IRSN

- **Examples of dose-response models used in PROTECT to estimate critical ecotoxicity values (i.e. EDR_{10} for chronic exposure).**



Step 2: Estimation of Expected No effect values (ENEVs)

ENEV for fish and mammals will be obtained by :

- **As a first approach, selecting the most sensitive CTV and applying an extrapolation factor to address the species-to-species extrapolation issue**
- **As a refined approach, incorporating the toxicity knowledge on each life history trait into dynamics population modelling for the species studied and estimating CTV for population-relevant endpoint - CTVpop (e.g., population growth rate) ;**
- **Under the assumption of constant sensitivity ranking among a taxonomic group, producing theoretical CTVpop at the population level for species exhibiting different life history traits;**
- **Analyzing the variation of sensitivity to alpha dose (rate) represented by the acquired sets of CTVpop among species**

Perform comparative dosimetry and radiochemistry of alpha-emitting radionuclides (e.g. Ra-226, Po-210) in fish and mammals (micro vs. macro effects, including the behavior of Rn-222). The focus should be on dose quantification for estimating and predicting higher-level organismal alpha effects

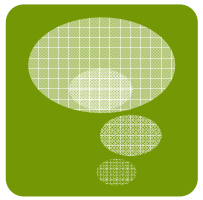
LAVAL

Toronto

McMaster

IRSN

McMaster University and Laval



- Dr Soo Hyun Byun has developed methods using windowless proportional counters for whole body estimations of dose.
- Dose distribution assays will be done at U laval (Dr Lariviere)

Phase 1 – Task 4.6

Perform comparative dosimetry and radiochemistry of alpha-emitting radionuclides (e.g. Ra-226, Po-210) in fish and mammals (micro vs macro effects, including the behaviour of Rn-222). The focus should be on dose quantification for estimating and predicting higher-level organismal alpha effects.

UL can do:

- Radiochemistry on biological samples for Ra-226 or Po-210, up to a maximum of 50 analyses. This number includes any duplicates, intakes sources, whole or partial body analysis.

- Dosimetry associated with the radiochemical analyses. Our approach will be compared to IRSN approach.

University of Toronto (Nareen Rahman)

- Study the behaviour of Rn-222 using methodology developed in Japan (lab)

Dosimetry

- Using the Po-210 data obtained through radiochemistry, whole-body internal dosimetry will be performed.

- Dose-conversion-factor (DCF) published by Amiro (*J. Environ. Radioact.*, 35:1 (1997) 37-51) will be used to calculate dosimetry to fish.

  - Po-210 : 2.73 x 10^{-5} Gy $year^{-1}$ per Bq kg^{-1} wet
  - Ra-226 : 2.46 x 10^{-5} Gy year-1 per Bq kg-1 wet

- Preliminary investigation of the distribution of Po-210 and Ra-226 in fish organs/tissues will also be performed on two individuals per radionuclide. This will provide a better assessment of heterogeneity associated with the biological distribution of radionuclides.

- Dosimetry study complemented by IRSN (second approach) using bio-kinetic models. McMaster can do measurements using proportional body counting.

Phase 2: Field Fish Experiments

Site Selection:

- An evaluation of available data on alpha-emitters in natural ecosystems will be conducted to identify appropriate reference and exposure conditions.
- In addition, results from controlled laboratory experiments will also be reviewed to identify concentrations at which potential effects were observed.
- Based on this information, appropriate sampling locations will be selected in consultation with CNSC staff and AREVA (Tamara).

Phase 2: Field Fish Experiments

Field Sampling:

- Field sampling of will be conducted at reference and alpha exposure sites to measure alpha-emitting radionuclides (e.g., Po-210) in water, sediments and fish.
- Concurrent sampling of effects data using the biomarkers measured in the Phase 1 laboratory experiments, where possible, as well as other relevant field measurements, as appropriate, will be conducted.

Phase 2: Field Fish Experiments

Reporting:

- Concurrent exposure and effects data that have been measured in the field will be analyzed to identify trends.
- Data will then be compiled for evaluation by IRSN to develop benchmarks.