

**EFFECTS OF ENDOCRINE DISRUPTING COMPOUNDS PRESENT IN  
FRASER VALLEY AGRICULTURAL RUNOFF ON EARLY LIFE  
STAGES OF RAINBOW TROUT (*ONCORHYNCHUS MYKISS*)**

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**EXTENDED ABSTRACT ONLY - DO NOT CITE**

**Introduction**

Agricultural manure runoff can contain both anthropogenic and natural endocrine disrupting compounds (EDCs). Anthropogenic sources include antibiotics and growth hormones, while natural sources include estrogen hormones and phytoestrogens. Natural sources become problematic when high density livestock or dairy operations such as those found in the Fraser Valley dispose of their manure to land. Given the high rainfall and water table in the Fraser Valley there is a high percentage of runoff generated from the application

of the manure to the fields. At particular risk to the manure runoff is the early life stages of salmonids that are spawned during the high precipitation fall/winter period. In order to determine the effect manure runoff has on the early life stages of fish, laboratory tests with rainbow trout (*Oncorhynchus mykiss*) and agricultural runoff were conducted each fall for three years (1999-2001) and in the winter of the first year.

### **Materials and Method**

*Field Procedures.* Agricultural plots on Seabird Island established by Agriculture and AgriFood Canada (1995) for Corn trials using Best Management Practices then turned over to grass in 1998 were used in the study. The plots are gently sloped (3-4%) with collection troughs and tanks at the end of each plot. Manure application rates and times coincided with the local agricultural community practices except that guidelines for manuring were complied with. Runoff was collected after each rain event following the final application of manure and transported to the laboratory. For the first two years bovine manure obtained from the University of British Columbia Agricultural Department was used on the plots, the third year hog manure from the local farming community was used.

*Laboratory Procedures.* Rainbow trout (*Oncorhynchus mykiss*) eggs and milt were obtained from a Department of Fisheries and Oceans (health) certified trout farm. Testing was conducted in accordance with the standardized Environment Canada test method for embryo/alevin/fry (EAF) (Environment Canada, 1998). Negative controls (well/dilution water, field plot runoff and solvent) and positive controls (nonylphenol and estradiol) were included in the study design. During the toxicity tests water quality parameters were measured. At specific time intervals, organisms were removed and preserved for gene expression profiling. Exposure concentrations for the bovine manure rain event diluted runoff were 100%, 50% and 25%. Due to the low dissolved oxygen content of the hog manure runoff (< 0.3 mg/L in 100%) a lower concentration series was required for this manure (25%, 10% and 1%).

### **Conclusions**

Salmonid early life stage bioassays conducted with two out of the three main sources of manure agricultural runoff (cow, hog, chicken) in the Fraser Valley indicate that manure runoff is lethal to rainbow trout, and that hog manure in

particular is extremely toxic to fish. The median effective concentration for nonviable embryos (EC50) for cow manure runoff was 65.4%, 70.7% and 54.2%, for years 1 fall, 1 winter and 2 fall, respectively. The runoff in the 1<sup>st</sup> year of the fall study with cow manure was most toxic to eggs (56% non-viability in 100% runoff, Figure 1), whereas in the 1<sup>st</sup> year winter and 2<sup>nd</sup> year the runoff was most toxic to the alevins. In the winter study all the alevins died within thirty-five days of total exposure to the runoff.

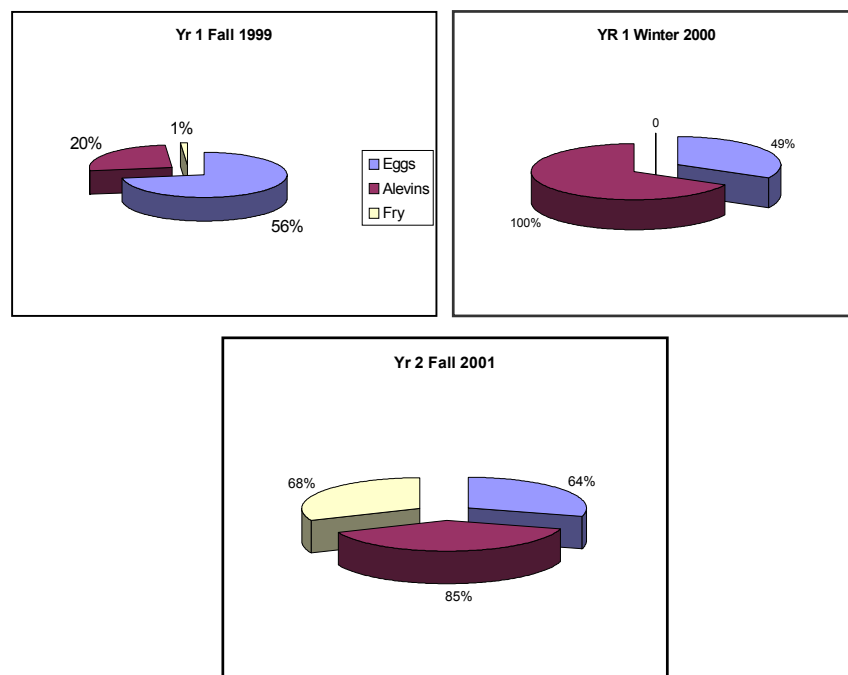


Figure 1. Toxicity of 100% Runoff from cow manured fields on different stages of rainbow trout

Runoff from hog manure was extremely toxic it had an EC50 of 2.7%. Greater than 50% of eggs hatched in the 25% and 10% runoff concentrations however, alevins were dead within a short period of exposure. Toxicity due to sediment contributed roughly the same amount of toxicity as the 50% cow manure runoff concentration,

approximately 36% of the test organisms died due to sediment smothering the eggs and clogging gills. For the hog manure exposures, sediment did not contribute to the toxicity.

<b>Compound</b>	<b>YR1 Fall 1999 COW</b>	<b>YR 1 Winter 2000 COW</b>	<b>YR 2 Fall 2000 COW</b>	<b>YR 3 Fall 2001 HOG</b>
Desmosterol	.02	.2	.02	<0.005
Cholesterol	1.0	13.0	.11	.2
Stigmasterol	.4	.4	.1	<0.005
Epicoprostanol	.1	.03	.01	.3
Coprostanol	.2	.03	.01	2.0
Beta-Sitosterol	.7	2.1	.2	.2
Equol	Not available	<0.2	<0.2	.05

Table 1. Concentration of Sterols in Manure Runoff ( $\mu\text{g/L}$ )

Table 1 indicates some of the sterols present in manure runoff. These EDC steroids may affect steroid metabolizing systems and alter hormonal balance and disturb normal reproductive function. Desmosterol is the precursor to cholesterol and stigmasterol in plants. Epicoprosterol is the precursor to coprosterol in bacteria, coprosterol is a reduced metabolite of cholesterol produced by bacteria in the intestinal tract of mammals such as hogs. B-sitosterol interferes with the cholesterol production pathway. Equol is an isoflavone found in alfalfa and soy, in animal systems equol inhibits aromatase which normally converts testosterone to estrogen.

The EDC positive controls, estradiol (25-50  $\mu\text{g/L}$ ) and nonylphenol (25  $\mu\text{g/L}$ ), like the manure runoff decreased hatching success, increased alevin mortalities, and increased non-viability at test end. Physiological effects included estradiol exposed alevins being paler and having poor skin quality, while nonylphenol exposed alevins were darker, smaller and had scoliosis. These spinal deformities enhance predation (Kruzynski and Birtwell, 1994). Dark pigmentation, either

indicating blindness or continuous spreading of melanophores due to chronic stress conditions and / or hormonal imbalance reduces the fish's chance of survival.

Gene analysis of the organisms exposed to estradiol and nonylphenol indicates an increased and accelerated expression of the  $\beta$ -estrogen receptor and vitellogenin compared with the solvent control, implying a loss of the normal regulation of gene control. Exposures with these EDCs indicate they disrupt early development at multiple levels of organization, ultimately to severely compromise the survival of the larvae. This study indicates early life stages of fishes are highly sensitive to agricultural runoff and EDCs which are potentially deleterious, either immediate or delayed.

### **Acknowledgements**

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### **References**

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