Fish Tumors Related to Great Lakes Areas of Concern Conference Proceedings

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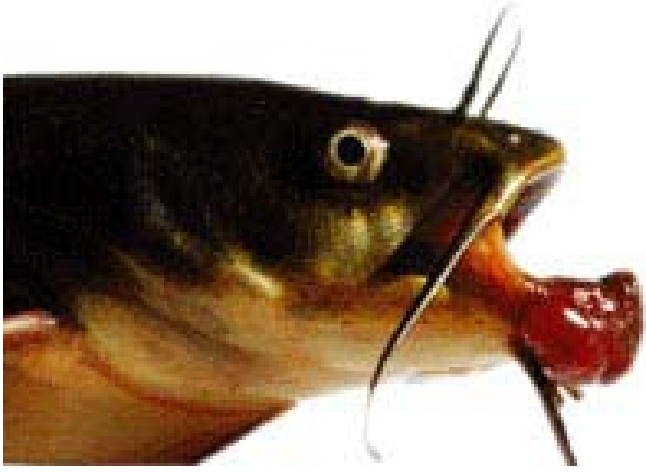

January 21 – 22, 2003
Erie, Pennsylvania

Compiled by: Sean Rafferty
Pennsylvania Sea Grant
Penn State Erie
5091 Station Rd
Erie, PA 16563-0501

Preface

The Fish Tumors Related to Great Lakes Areas of Concern Conference Proceedings was compiled with the intention of capturing the thoughts of the conference held in Erie, Pennsylvania from January 21-22, 2003; as well as to provide information on fish tumors as they relate to the beneficial-use impairment in Areas of Concerns. Two working subcommittees, monitoring and histopathology have been formed as an outcome of the conference. They are being chaired by Paul Baumann (monitoring) and Vicki Blazer (histopathology), and are preparing standardized criteria for this beneficial-use impairment to be used in all Areas of Concern addressing this use impairment. The resulting work of the subcommittees will be presented at a follow up conference on August 18-19, 2003, at Penn State Erie. The August conference attendees will attempt to complete their recommendations of standardized criteria for fish tumors and deformities and submit a concept paper to the International Joint Commission at its September meeting in Ann Arbor, Michigan.

A special thanks is extended to all the speakers at the conference, including Dr. Paul Baumann, Dr. Vicki Blazer, Kelly Burch, Dr. John (Jack) Fournie, Dr. John Gannon, Dr. John Harshbarger, Chuck Murray, Dr. Fred Pinkney, Roger Thoma, and Bob Wellington, and also to the U.S. Environmental Protection Agency and the Pennsylvania Department of Environmental Protection for providing funding for the conference. We would also like to extend our thanks to Gannon University for hosting the conference.

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Contacts:
For additional information on fish tumor concerns, conference, or on follow-up activities, please contact:

Eric C. Obert
Associate Director
Pennsylvania Sea Grant
Penn State Erie
Erie, PA 16563
(814) 898–6453
eco1@psu.edu

Lori Boughton
Chief, Office of the Great Lakes
PA Department of Environmental Protection
230 Chestnut Street
Meadville, PA 16335
(814) 332-6816
lboughton@state.pa.us

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Introduction – Conference Objectives

The International Joint Commission (IJC) characterized fish tumors and other deformities as one of 14 beneficial-use impairments to be used by Areas of Concern as criteria for the listing and delisting process. An Area of Concern is considered to have a fish tumor and deformity impairment if the following criteria are observed.

1) An intestinal or liver tumor prevalence of $\geq$ 5 to 7% occurs in common native nearshore species of benthic dwelling fish (brown bullhead), walleye, perch or salmonid species offshore. Samples must consist of 30 fish, each of which is 250 mm or greater in length. Tumors are defined as neoplasms of either intestinal, bile duct, or liver cells as determined by histopathology
2) A prevalence of lip tumors $\geq$ 8-10% or overall external tumors $\geq$ 13-15% in white sucker and brown bullhead. Tumors are defined as papillomas or other neoplasms as determined by histopathology. Samples must consist of at least 30 fish, each of which is 250 mm in length or greater.
3) A Deformities, Erosion, Lesions & Tumors (DELTs) external anatomy index of > 0.5% occurs. (Baumann; LaMP, 2000)

Tumors in various fish species, predominately brown bullheads, in Presque Isle Bay and other Areas of Concern have concerned researchers and citizens since the creation of Areas of Concern on the Great Lakes in 1984. In order to refine and coordinate the standardization of protocols currently being used to evaluate this beneficial-use impairment, staff of Pennsylvania Sea Grant, the Pennsylvania Department of Environmental Protection (DEP), and the U.S. Environmental Protection Agency (EPA) worked together to coordinate a conference addressing fish tumors in Areas of Concern.

On January 21–22, 2003, Pennsylvania Sea Grant, the DEP, and the EPA co-sponsored the first Area of Concern Conference on Fish Tumors, held at Gannon University in Erie, Pennsylvania. This conference brought together more than 40 researchers, fishery and wildlife biologists, pathologists, and agency representatives. The goal of the conference was to share information, concerning fish tumors and deformities, from American and Canadian Areas of Concern, and to refine and coordinate the standardization of protocols currently being used to evaluate this beneficial-use impairment. Organizers wanted to develop criteria for this beneficial-use impairment based on the recommendations of the participants. During the two-day conference several panel discussions were held in order to address concerns in relation to establishing criteria for analyzing fish tumors and deformities. Many questions and concerns were left unresolved; however, the conference was the first step in establishing a functioning network of scientists to collaborate on research issues concerning fish tumors and deformities, and develop standardized criteria for the analysis of this beneficial-use impairment.

Speakers and conference participants helped set the stage for many of the panel discussions by submitting preconference questions on several topics that required the need for standardization. Presenters and participants proposed the following questions and concerns regarding this beneficial-use impairment.

The need for histology of internal and external lesions:

- How much liver or what parts of the liver should be sampled?
- How many similar external lesions should be sampled?
- Should "normal" skin sections be sampled?
- Should internal organs, aside from the liver, be sampled for deformities?

How tumors are diagnosed; standardization of criteria used in naming deformities:

- Criteria for cellular alteration vs. hepatoma vs. hepatocellular carcinoma?
- Criteria for bile duct cell neoplasms vs. cellular proliferation?
- Criteria for papillomas vs. carcinomas?
- Diagnosis of pigmented skin lesions?

The relationship of age to tumor occurrence, including:

- Minimal age restrictions to allow for exposure and latent periods?
- Balancing numbers needed vs. accuracy in deciding to use single age comparisons?
- Looking at how neoplasms of differing stages relate to age, do they all follow the same pattern?
- Use of spines vs. otoliths for aging; how accurate are spines for older ages?
- Is there a source for bullheads of known age? If not, who can culture them?

Statistical considerations:

- Occurrence of lesions vs. counts of lesions?
- Use of most advanced lesion vs. using combined stages?
- Is a neoplasm index possible using counts, stages, and/or areas?
- Level of probability needed?

It is the hope of conference organizers that the work and collaboration that began at the conference will lead to the development of standardized criteria concerning fish tumors and deformities.

Background Information

The International Joint Commission (IJC) was formed in 1909, comprised of American and Canadian officials, to assist these governments in finding solutions to the problems facing the waters bordering the United States and Canada, and to manage and protect these waters for the benefit of today's citizens and future generations.

In 1987, the U.S. and Canadian governments signed a protocol promising to report on the progress associated with the improvement of Areas of Concern and requiring the IJC to review Remedial Action Plans (RAPs). Areas of Concern are described as geographic areas, within the Great Lakes Basin, that fail to meet the general or specific objectives of the agreement where such failure has caused or is likely to cause impairment of beneficial use of the area's ability to support aquatic life.

Remedial Action Plans are being developed and implemented at the 42 current Areas of Concern. The objective of the RAP is to restore the beneficial uses, as identified in Annex 2 of the Great Lakes Water Quality Agreement (GLWQA). The mechanisms responsible for the loss of ecological integrity in Areas of Concern are identified in the first step of the RAP development process. Plans of action are then designed to rejuvenate these areas to levels that meet government and public expectations. The restorative measures use an ecosystem approach which considers not only land, air, and water degradation; but also the loss or restriction of human uses in the Great Lakes Basin.

The focus of the conference was fish tumors and other deformities in relationship to Areas of Concern. Tumors can be defined as a swelling on some part of the body; whereas, the swelling or lump represents an abnormal growth of new tissue and the new tissue differs in appearance from the surrounding tissue. Tumors and deformities affecting the brown bullhead (indicator species for this beneficial-use impairment) are hypothesized to be the result of viruses, parasitic invasion, hybridization, and contaminated sediment.

Brown bullheads are commonly affected with epidermal neoplasms of the mouth and skin, where neoplasms are defined as an abnormal tissue that grows by cellular proliferation to form a distinct mass of tissue that may be benign or malignant. The neoplasms are diagnosed as either papillomas (benign) or carcinomas (malignant) and occur singly or in multiples varying in size from several millimeters to several centimeters. When taking a tissue sample of a skin tumor, the entire deformity along with a portion of the underlying tissue should be removed. Tumors in brown bullheads normally originate from two cell types: lightly pigmented neoplasms are usually composed of neoplastic epithelial cells, and black to dark brown growths are usually composed of neoplastic pigment cells. Brown bullheads have exhibited variably sized irregular areas of superficial, dark brown to black pigmentation known as melanosis.

In brown bullheads, lesions include healing wounds and ulcers; excessive scar tissue accumulations around old injuries, especially in the mouth; abrasions from sampling gear; bacterial infection; epidermal hyperplasia; and injuries from pectoral and dorsal spines.

Neoplasia of hepatocytes (liver cells) is termed hepatic cell adenoma (non-invasive, benign) or hepatic cell carcinoma (invasive, malignant). Neoplasia of the bile duct is diagnosed as cholagioma (benign) or cholangiocarcinoma (malignant). Grossly, cholangiomas may appear as white or cream-colored foci or nodules that may be several centimeters in diameter. Early stage neoplasms of hepatocellular origin may be similar to the bile duct tumors in gross appearance, and more advanced tumors may appear as white, gray, cream-colored, or reddish-tan masses bulging from or as nodules within the liver tissue.

Conference Summary

The purpose of the fish tumor conference was to bring researchers and agency staff together to develop standardized criteria for the analysis of fish tumors and other deformities in Areas of Concern. The objectives were to determine what is currently being done in the analysis of fish tumors and deformities, and develop a uniform system for this beneficial-use impairment.

History

The Great Lakes Water Quality Agreement was first signed in 1972, in which the United States and Canada agreed to restore and preserve the chemical, physical, and biological integrity of the Great Lakes Basin ecosystem. In 1978, a new agreement was reached, in which both countries pledged a commitment to rid the Great Lakes of persistent toxic substances (substances that remain in the environment for long periods of time, poisoning food sources for animals and humans). In 1987, a Protocol was signed by both governments, promising to report on restorative progress and calling on the International Joint Commission to review Remedial Action Plans proposed by the 42 Areas of Concern. The mission of the Remedial Action Plans is to restore beneficial uses as identified in Annex 2 of the Great Lakes Water Quality Agreement, in degraded areas within the basin.

Current Knowledge

A fish tumor or deformity impairment occurs when incidence rates of tumors and/or deformities exceeds the specified rate at un-impacted control sites or when data verifies the presence of neoplastic or preneoplastic liver tumors. Un-impacted sites are areas where there is a lack of industrial or municipal pollutant discharges located upstream or in the immediate area where neighboring land uses have not disrupted ecosystem function. Bullheads and suckers are considered inshore fish species and are not known to extensively migrate; therefore, the health of these species can be used to assess the impacts of localized aquatic environments on the health of fish species.

The purpose in assessing fish tumors and deformities is to use these as an indicator of environmental degradation of the aquatic ecosystem and a measure of health impairment to fish populations. Tumors are defined as heritably altered, independent (meaning functions outside host), relatively atypical tissue growths. Tumors can be induced genetically, virally and chemically. Deformities are defined as twisted, missing, forked, or bulging body parts including deformed fins, barbels, abdomen, or skeleton. Deformities are caused by several factors, including: environmental degradation (e.g. chemical contaminants), rapid temperature change during early development, viruses, bacteria, and parasites. Lesions are open sores, exposed tissue, and/or prominent bloody areas.

Currently, 16 of the 42 (Collingwood Harbor was delisted in 1994) Areas of Concern have impairment of beneficial uses due to the presence of fish tumors and other deformities. Those included are: Ashtabula River, Ohio; Black River, Ohio; Buffalo River, New York; Cuyahoga River, Ohio; Detroit River, Michigan; Grand Calumet River, Indiana; Maumee River, Ohio; Milwaukee Estuary, Wisconsin; Niagara River, New York; Presque Isle Bay, Pennsylvania;

Rouge River, Michigan; Sheboygan River, Wisconsin; St. Louis River and Bay, Minnesota and Michigan; St. Mary's River, Michigan; Thunder Bay, Ontario; and Jackson Bay, Ontario.

Future Focus:

- Standardize the fish tumor and other deformities sampling protocol for Great Lakes' Areas of Concern.
    - Establish minimum criteria: sample size, age, length, gender, year, etc.
    - Which organs are to be analyzed for tumors and other deformities
    - The number of slides needed for histopath analysis of samples
    - Data needs for the proposed database
    - How many and what sites should be sampled within an Area of Concern
    - DELT versus histopathological analysis

- The frequency at which fish are sampled within an Area of Concern needs to be determined

- The use of otolith or spine analysis to age fish

- Bullhead migratory patterns (are they resident or do they migrate)

- Standardization of fish tumor and deformity criteria

- Development of a list of causes for fish tumors and other deformities

- Comprise a chart that depicts the various tumor types

- Investigate the possibility of a central data repository for Great Lakes Areas of Concern

- Establish task forces and committees to ensure that all concerns in regard to fish tumors and other deformities are addressed and to investigate funding for the assessment of beneficial-use impairments in Areas of Concern

Presentations – Keynote Address

Kelly Burch

Mr. Burch began the address by thanking Pennsylvania Sea Grant and the U.S Environmental Protection Agency for providing the funding for the Fish Tumor Conference. He then provided an overview of the history of Presque Isle Bay, as it relates to fish tumors. Presque Isle Bay was designated as an Area of Concern in 1991, representing the last Area of Concern to be listed. Out of the 43 Areas of Concern, Presque Isle Bay is the only to be listed because of community involvement as opposed to government recommendation, and was designated because of the presence of tumor-containing fish and contaminated sediment.

Following Presque Isle Bay's new designation, a Remedial Action Plan was developed to restore and manage the health of the bay. In 1992, a tumor study was conducted in Presque Isle Bay and the results determined that: 64% of bullheads contained external tumors and 22% contained liver tumors. In 1992 and 1993, bullheads were tagged to determine migratory behavior, population estimates, and identification for recapture. The Department of Environmental Protection determined that in order to eliminate the problem facing the health of Presque Isle Bay, both point and nonpoint sources of pollution needed to be eliminated prior to any consideration of dredging activity. Since its designation as an Area of Concern, more than $100 million has been spent building more efficient sewage treatment facilities and eliminating 60-65 sewage overflows, which affected Presque Isle Bay.

One of the concerns facing researchers involved with restoring Presque Isle Bay was the development of a uniform protocol for determining the age of effected bullheads; use of otoliths versus the use spines? In order to restore and manage a designated Area of Concern, goals and targets need to be set for each beneficial-use impairment at hand. Several of the Canadian Areas of Concerns have developed such protocols, and the American Areas of Concern are now beginning to adopt the use of goals and targets.

Mr. Burch concluded the keynote address with several quotes, including: "if it cannot be measured it cannot be managed," "Great Programs, Great People, Great Lakes," and "Thanks for making the Lakes Great!"

Presentations – Session I: Historical Overview

Dr. John Harshbarger - Overview of Fish Tumor History and Epidemology

Dr. Harshbarger opened his presentation by clarifying the following terms: tumor, neoplasm, toxin, and hyperplasia. Tumor and neoplasm are interchangeable in current medical usage. A tumor or neoplasm, is a heritably altered (mutated), relatively independent (autonomous), relatively atypical (dysplastic) growth of tissue of no use and often detrimental to the host. In other words a neoplasm is a population of abnormal cells that continue to proliferate after mutation is no longer present. Tumors that are growing by simple expansion are often benign while tumors that are invading and destroying host tissue are cancers. Since cancers can arise in benign tumors one should not become complacent about benign tumors. Causes of the heritable abnormality include certain chemicals, ionizing radiation, ultraviolet radiation, and certain viruses. There is no minimum threshold level for the oncogenic mutagen.

Toxin is derived from the Latin word *toxicum* meaning poison. Poison kills cells via the production of free radicals that interfere with intracellular mechanisms: thus, toxin causes the cessation of cellular-proliferation in contrast to neoplastic transformation, which enhances cellular proliferation. Toxins have a minimum threshold.

Hyperplasia is the unscheduled proliferation of normal cells and is often accompanied by organ hypertrophy. Examples include: 1) Kidney donors have compensatory hyperplasia and hypertrophy of the retained kidney; 2) Overeaters have nutritional hyperplasia of adipose tissue to store the excess calories; 3) Hypertension induces functional hyperplasia and hypertrophy of cardiac muscle; 4) Sunburn releases toxic free radicals leading to regenerative hyperplasia to replace the dead skin cells; 5) Iodine deficiency biofeedback causes endocrine hyperplasia of the thyroid tissue with goiter formation.

Following the clarification of these terms, Dr. Harshbarger briefly outlined the history of the role of carcinogens in tumors and milestones in the use of fish environmental sentinels.

- 1775: Sir Percival Potts reported that boys used as chimney sweeps developed scrotal cancer.
- 1850: Fish neoplasms first documented in North America.
- 1900: The carcinogenicity of coal tar (chimney soot) was experimentally confirmed.
- 1930: Benzo(a)pyrene was the first pure carcinogen isolated from coal tar.
- 1940: Skin papillomas were discovered on brown bullheads in industrially polluted Delaware and Schuylkill Rivers in Philadelphia.
- 1956: Evidence that a herpesvirus can cause cancer was discovered in northern leopard frogs.
- 1957: Oral papillomas were found on the lips of white croakers feeding at a California sewage outfall while white croakers feeding in relatively pristine water were tumor free.

- 1962: The carcinogenicity of aflatoxin, a common, potent human carcinogen produced by fungi, was discovered when hatchery rainbow trout developed panzootic liver cancer following the global introduction of mold contaminated pelleted trout chow.
- 1963: White suckers in a polluted waterway had oral papilloma and liver cancer.
- 1964: Zebrafish were exposed to diethylnitrosamine in the first experimental carcinogen study with small fish.
- 1965: The Registry of Tumors in Lower Animals was started.
- 1970: The 1940's report of skin cancer was confirmed and liver cancer was discovered in brown bullhead catfish sampled every 10 miles in the Delaware River between Trenton, NJ and Philadelphia, PA.
- 1972: Neoplasms reported in several fish species in the polluted Fox River west of Chicago compared to almost none in the same species from pristine Canadian lakes.
- 1977: Liver cancer reported in English sole in a polluted tributary of Puget Sound.
- 1978: Tomcod liver cancer discovered in lower Hudson River, NY heavily polluted by PCB's and PAH's.
- 1979: Paul Baumann found skin and liver cancer in brown bullheads at a coking plant outfall in the Black River, Ohio.
- 1981: Neoplasms reported in fish species from the Buffalo River, Buffalo NY.
- 1981: Liver cancer reported in sauger and walleye from Torch Lake MI contaminated by copper mine tailings and chemicals used to extract copper.
- 1982: CNN ran a series of reports on fish tumors associated with chemicals dumped into Torch Lake (Michigan), the coking plant on the Black River and the lower Hudson R. There was a huge unexpected worldwide response.
- 1983: Congressional hearing held in concern of fish cancer prevalence where human cancer was also high.
- 1985: Winter flounder liver cancer reported from Deer Island sewage outfall, Boston Harbor.
- 1985: Skin painting of extracts of sediment from Black River, OH and Buffalo River, NY produced cancer on brown bullheads and mice.
- 1987: Bowfin liver cancer reported from Detroit River, MI.
- 1987: White perch liver tumors reported from Chesapeake Bay.
- 1988: Oyster toadfish pancreas and liver cancer reported from York River, VA near a refinery.
- 1988: Oral papillomas and liver neoplasms in white sucker reported from polluted sites on Lake Ontario
- 1990: Mummichog liver cancer reported from creosote polluted Elizabeth River, VA.
- 1991: Experimental trophic transfer of carcinogens to winter flounder fed contaminated blue musssels
- 1991: Brown bullhead liver cancer reported in Cuyahoga River, Cleveland, OH.
- 1995: Brown bullhead liver cancer in Black River, OH drops sharply after coking plant closes and PAH's plummet.
- 1995: Oral papilloma reported in white sucker from St Lawrence River, PQ Canada
- 1998: Lake whitefish liver cancer reported in St Lawrence River, PQ Canada.
- 2001: Brown bullhead liver and skin cancer reported in the Potomac River, and Anacostia River

This incomplete list shows the importance of fish liver and skin neoplasms as sentinels for environmental carcinogens. Dr. Harshbarger discussed epizootic tumors in other organ systems as well including the hematopoietic system, pigment and nerve cell neoplasm, excretory system, etc., according to the predominate species and site of their occurrences. The number of epizootic fish, amphibian, reptile, and mollusk neoplasms has increased steadily from a combined total of 18 in 1954 to 145 in 1994.

Several examples were given linking pollutants and chemical contaminants to tumors and deformities in fish species associated with affected environments. These included mid-western frogs with polydactyly (Fig 1) and sea lampreys in the Great Lakes and tributaries with teratoid anomalies (Fig 2). In Orange County, California, oral papillomas in white suckers near the sewage outfall (Fig 3) declined to zero following the renovation of the sewage facilities.

Dr. Harshbarger was an expert witness at a trial concerning Millstone nuclear power plant in Connecticut and its involvement in the massive discharge of carcinogenic compounds into the surrounding aquatic ecosystem. Nuclear power plant operators add huge volumes of chemical oxygen scavengers such as hydrazine to the cooling water to prevent internal corrosion. The principle compounds used are carcinogenic and associated with carcinogenic contaminants; therefore, the large number of nuclear power plants around the great lakes are a likely source of environmental carcinogens and fish in the vicinity of the discharges should be monitored for liver cancer.

Dr. Harshbarger was asked by the IJC to put together a report relating contaminants and fish tumor occurrence. It was his suggestion that all sources of point source pollution should be documented and the chemicals that were being put into the environment should be noted. Bioassays of fish containing tumors and deformities could be carried out to determine if the chemical causing these deformities could be linked to point source pollution. He then concluded by stating: “the companies or persons responsible for putting these harmful pollutants into the environment should be responsible for their actions, and if they were not, penalties should be administered.” Selected data from that report are included here.

Dr. John Harshbarger's Power Point Presentation

Figure 1: Frog With Polydactyly

Figure 2: Histopath of a Teratoid Anomaly Found in Sea Lampreys

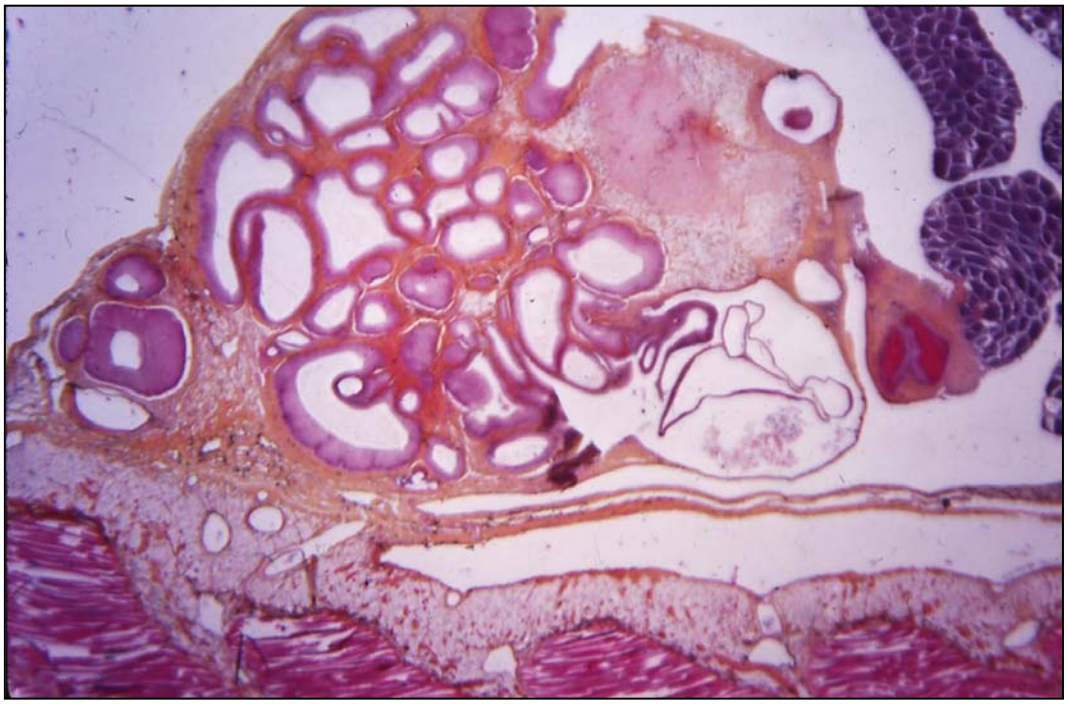

Figure 3: Oral Papillomas on a White Sucker

Toward a Transboundary Monitoring Network:
A Continual Binational Exploration

Vol. 2

Proceedings of a Workshop Convened by the International Joint Commission, U.S.A. and Canada

And

Relata Assembled During the Editing Process

Bruce L. Bandurski, Peter T. Haug, and Andrew L. Hamilton
Editors

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Table II
Fish Used in Experimental Carcinogenesis

Atheriniformes		
Atherinidae		
	Menidia beryllina	Inland silverside
Cyprinodontiformes		
Aplocheilidae		
	Rivulus marmoratus	Rivulus
Cyprinodontidae		
	Cyprinodon variegatus	Sheepshead minnow
	Fundulus grandis	Gulf killifish
Oryziidae		
	Oryzias latipes	Medaka
Poeciliidae		
	Poecilia formosa	Amazon molly
	Poecilia reticulata	Guppy
	Poeciliopsis lucida	Topminnow
	Poeciliopsis monica	Topminnow
	Xiphophorus helleri	Green swordtail
	Xiphophorus maculatus	Southern platyfish
	Xiphophorus montezumae	--
	Xiphophorus xiphidium	--
	Xiphophorus variatus	Variable platyfish
Cypriniformes		
Cyprinidae		
	Danio rerio	Zebra danio
	Pimephales promelas	Fathead minnow
	Rhodeus aceletus	Bitterling
Gasterosteiformes		
Gasterosteidae		
	Gasterosteus aculeatus	Threespine stickleback
Salmoniformes		
Salmonidae		
	Oncorhynchus nerka	Sockeye salmon
	Salmo gairdneri	Rainbow trout
	Salmo trutta	Brown trout
	Salvelinus fontinalis	Brook trout
Siluriformes		
Ictaluridae		
	Ictalurus nebulosus	Brown bullhead
	Ictalurus punctatus	Channel catfish

Table III
Chemicals Used in Experimental Fish Carcinogenesis

Aflatoxins	
AFB_1	Aflatoxin B_1
AFG_1	Aflatoxin G_1
AFM_1	Aflatoxin M_1
AFQ_1	Aflatoxin Q_1
AFL^1	Aflatoxicol
AFL^1	Aflatoxicol
Sterigma	Sterigmatocystin
VCN	Versiculorin A
Aromatic Amines	
AAT	O-aminoazotoluene
DAB	4-dimethylaminoazobenzene
AAF	2-acetylaminofluorene
Nitrosamines	
DMN	Dimethylnitrosamine
DEN	Diethylnitrosamine
DNNM	2,6-dimethylnitrosomorpholine
DNP	N-dinitrosopiperazine
NM	N-nitrosomorpholine
NPYR	N-nitrosopyrrolidine
MNU	N-methyl-N-nitrosourea
MNNG	N-methyl-N'-nitro-N-nitrosoguanidine
Polycyclic Aromatic Hydrocarbons	
Anthracene	Anthracene
BP	Benzo[a]pyrene
DMBA	7,12-dimethylbenz[a]anthracene
MC	3-methylcholanthrene
Others	
EMS	Ethyl methanesulfonate
MAMA	Methylazoxymethanol acetate
BRKN	Bracken
CPFA	Cyclopropanol fatty acids Sterculic acid Malvalic acid
DDT	Dichlorodiphenyltrichloroethane
DEPH	Di-2-ethylhexyl phthalate
EDB	Ethylene dibromide (1,2-dibromoethane)
NP	Nifurpirinol

Relevance of Fish Cancer to Human Cancer

Common Basis

Many human cancers are believed to be due to altered activity of 20 or so host growth factor genes or oncogenes. Data has been published for similar oncogenes in fish and in various invertebrates down to the primitive level of sponges. Thus, many types of cancer appear to have a common basis throughout phylogeny.

Common Metabolism

Most carcinogens act indirectly, that is, they are not carcinogenic themselves, but when they are metabolized for excretion, usually by the liver, reactive, proximate, carcinogenic intermediate compounds are created. Fish utilize metabolic pathways similar to mammals in the process.

Common Results

Experimentally, mammalian carcinogens are also carcinogenic for fish and the liver is the primary target organ for most chemicals in both cases.

Carcinogen Bioassay

Pure test material can be microinjected directly into Mt. Shasta strain rainbow trout ova at a rate of 200 ova/hr/person. This combines a well-known sensitive fish having a 20-year record of carcinogenecitity studies with the most sensitive stage (embryo). It uses the least amount of chemical in a closed route of exposure for maximum safety and minimum by-product for disposal. In lieu of injection, ova can be bathed in the test chemical for 15 minutes. Liver tumors begin appearing in three months and 12 months is the usual post exposure period.

Also, the ova bathing exposure can be utilized with small fish species that appear especially suitable for carcinogen bioassay. Medaka appears to be the best small fish species for bioassay, but several others are also promising, including guppy, rivulus, platyfish/swordtail hybrid, zebra danio, topminnow and Amazon molly. Small fish have the advantage that a sagittail section of the entire fish will fit on a single micro slide for expeditious examination of all tissues. A second advantage is that liver tumors begin appearing in seven weeks; therefore, six months is a suitable post treatment period.

Advantages of Fish Bioassay

(1) Miniscule amount of test chemical for safer handling and disposal.

(2) High sensitivity, equivalent or better than rodents and significantly, this based on a single short exposure.

(3) Six months to one-year experimental period versus two years for rodents.

(4) All or none response. No tumor in controls, as often happens in rodent experiments requiring statistical evaluation to significant difference.

(5) Cost: Approximately $20,000/test versus $500,000 to $1,500,000 for rodents.

(6) No sentimental lobby groups protesting cruelty to fish.

(7) Useful to test carcinogenicity of chemical mixtures in concentrated effluent, sediment extracts and extracts of smokestack filtrate. Can also be used to bioassay bile extracts of wild fish and liver equivalents of wild invertebrates for carcinogenic reactive metabolic intermediates.

(8) Fish are real world organisms, i.e., they are part of the natural ecology rather than being inbred laboratory animals.

Conclusions

One necessary step to clean up the environment is to eliminate the input of noxious chemicals. Eventually, as shown by declining DDT levels in the Great Lakes, microbial and other types of degradation will gradually reduce residues. The only way to stop input of noxious chemicals into the environment is to register every outfall and smokestack, test the output regularly for noxious chemicals, penalize owners for non-compliance and make owners fully responsible for resulting detrimental effects.

It is proposed that chronic fish bioassays of effluent are an efficacious method to detect carcinogens and teratogens in outfall effluent concentrates and in smokestack filtrates. Rodent bioassays of effluent are too costly and time consuming for broad chronic carcinogenic screening, but positive results of fish bioassay could be funneled to rodent tests for corroboration if fish results were challenged (so far rodents have been little used for bioassays of chemical mixtures). In addition to testing effluent, the chronic fish bioassay is useful in: (1) testing bile from wild fish or extracts from liver equivalents of wild invertebrates to determine presence of carcinogens in water ways and (2) testing new or untested existing chemicals to prevent or eliminate exposures to unsuspected carcinogens in common usage.

Dr. John Gannon – Fish Tumor Listing/ Delisting Criteria

Dr. Gannon began his presentation by discussing how the problems facing the health of fish species arose and what needs to be done to restore fish health. The appearance of fish tumors and other deformities are believed to have appeared with the onset of the industrial revolution. In order to restore fish health, sources of pollution must be eliminated and aquatic habitats must be restored through human intervention (i.e. dredging and excavation) and/or natural recovery.

Dr. Gannon followed the fish health issues by outlining the history of the binational management policy. In 1972, the Great Lakes Water Quality Agreement (GLWQA) was developed in order to decrease phosphorus concentrations in the hope of preventing eutrophication. The GLWQA was revised in 1978 to include toxic substances. Areas containing toxic substances were separated into two classifications: Class A – severely polluted and Class B – moderately polluted. A protocol was designed in 1987, which included the designation of Areas of Concern and the development of Remedial Action Plans in order to restore these areas.

In 1988, listing and delisting criteria were developed in order to restore the 43 Areas of Concern (currently there are 42 Areas of Concern – Collingwood Harbor in Canada has been delisted). These criteria are known as beneficial-use impairments and are related to both human activity and ecosystem impacts. There are currently 14 beneficial-use impairments:

1) Restrictions on drinking water consumption, or taste and odor problems
2) Beach closings
3) Degradation of aesthetics
4) Added costs to agriculture of industry
5) Restrictions on fish and wildlife consumption
6) Tainting of fish and wildlife flavor
7) Restrictions on dredging
8) Eutrophication or undesirable algae
9) Degradation of phytoplankton and zooplankton populations
10) Degradation of benthos
11) Degradation of fish and wildlife populations
12) Loss of fish and wildlife habitat
13) Bird or animal deformities or reproduction problems
14) Fish tumors or other deformities

Dr. Gannon went into detail discussing the beneficial-use impairment: fish tumors or other deformities. When bullhead liver tumors exceed 2% or 3.5% in suckers, it is listed as a beneficial-use impairment, and when rates drop below these levels it is delisted. Dr. Gannon proposed the question, "does this value need to be updated to reflect current trends or new scientific evidence?"

Dr. Gannon concluded his presentation by proposing challenges and opportunities that need to be addressed as they relate to the science linkage and science-management linkage.

Science linkage

- Cause and effect links between fish tumors and environmental contaminants
- Population and ecosystem response to remediation (i.e. changes in biodiversity)
- Habitat creation, restoration, and protection of soft sediments or "soft engineering" of hard substrate (alternatives to rock or steel rip-rap)

Science-Management linkage

- Monitoring, assessment and evaluation component
- Guidance on Area of Concern fish tumor abnormality studies based on case studies in the Black River and other areas
- Refine listing/delisting criteria for fish tumors

Dr. John Gannon's Power Point Presentation

The Fish Tumor Listing / Delisting Criterion

Its History and Prognosis for the Future in Linking Science and Management in the Great Lakes Areas of Concern

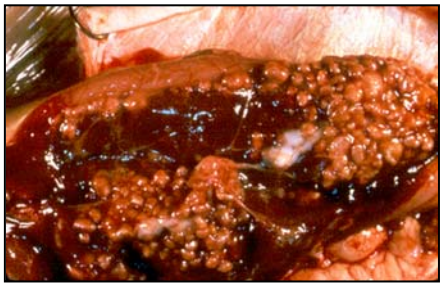
By Dr. John Gannon
Great Lakes Regional Office
International Joint Commission
Windsor, Ontario

Fish Health Problems:

Just One Expression of the

Loss of Ecosystem

Integrity



How Did We Get Into This Mess?

How Do We Get Out of This Mess?

- **Eliminate Sources of Pollution**

- **Remediate and Restore Habitat Through**
  - *** Human Intervention**
  - *** Natural Recovery**

History of Binational Resource Management Policy Response

1972 – Great Lakes Water Quality Agreement (GLWQA): Focus on Eutrophication of Phosphorus Control.

1978 – GLWQA: emphasis on Toxic Substances
- **Class A Areas of Concern (Severely polluted)**
- **Class B Areas of Concern (Moderately polluted)**

1987 – GLWQA Revision by Protocol
- **42 Areas of Concern**
- **The "How Clean is Clean?" Debate**
- **Evolution of Listing/De-listing Criteria**

2001 – Recognition of "Area of Recovery"

14 Beneficial Use Impairments

- **Restrictions on drinking water consumption, or taste and odor problems**
- **Beach closings**
- **Degradation of aesthetics**
- **Added cost to agriculture or industry**
- **Restrictions on fish and wildlife consumption**
- **Tainting of fish and wildlife flavor**
- **Restrictions on Dredging**
- **Eutrophication or undesirable algae**
- **Degradation of phytoplankton and zooplankton populations**
- **Degradation of benthos**
- **Degradation of fish and wildlife populations**
- **Loss of fish and wildlife habitat**
- **Bird or animal deformities or reproduction problems**
- **Fish tumors or other deformities**

Fish Tumors or Other Deformities

Listing Criteria

When the incidence of neoplastic or pre-neoplastic liver tumors exceeds 2% in bullheads or 3.5% in suckers.

De-Listing Criteria

When the incidence of neoplastic or pre-neoplastic liver tumors in bottom-dwelling fishes does not exceed 2% in bullheads or 3.5% in suckers.