



## Project Summary

# Causes of Papillomas on Fish Living in Chlorinated Sewage Effluent

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This research was initiated to determine the cause of oral papillomas on black bullheads (*Ictalurus melas*) from the final oxidation pond of the Tuskegee, Alabama, sewage treatment plant. The water in this pond was chlorinated effluent from the sewage treatment plant. Ames-test mutagenicity of a pond-water concentrate indicated the presence of a chemical carcinogen in the pond water. However, water and sediment analysis did not identify substances suspected of causing the tumors. Cytoplasmic inclusion bodies were present in some papilloma cells, but attempts to find virions in the tumor via electron microscopy and to transmit the papillomas by means of injection of cell-free tumor homogenate into black bullheads were not successful. Juvenile black bullheads, yellow bullheads (*Ictalurus natalis*), and channel catfish (*Ictalurus punctatus*) were confined to cages in the oxidation pond and in a control pond. Most caged black bullheads in the oxidation pond developed focal, oral hyperplasia and stomatitis in the same mouth locations occupied by papillomas in wild black bullheads from this pond. These hyperplastic lesions healed in most fish during additional exposure. Mucosal hyperplasia also occurred, but at a much lower incidence, in other test species and in all control species. A similar incidence of hyperplastic lesions in black bullheads in floating and sunken cages indicated that contact with the sediment or ingestion of benthic food organisms did not affect pathogenesis of the lesion.

*This Project Summary was developed by EPA's Environmental Research Lab-*

*oratory, Gulf Breeze, FL, to announce key findings of the research project that is fully documented in a separate report of the same title (see Project Report ordering information at back).*

### Introduction

A population of black bullheads in the 1.0-hectare, final oxidation pond of the Tuskegee, Alabama, sewage treatment plant had a 70% prevalence of oral papillomas.<sup>1</sup> These fish provided an opportunity to study the etiology of fish papillomas, especially as related to chlorinated sewage effluent.

Sewage entering this treatment plant did not include discharge from industries or institutions, but run-off water from residential and agricultural areas entered the plant after rains. The chlorination treatment of sewage after it passed through an aeration basin and a settling basin was considered a possible source of carcinogens.

The only previous report of a high prevalence of neoplasms in aquatic animals living in chlorinated sewage effluent involved a pond at Reese Air Force Base, Texas. In that case, tiger salamanders (*Ambystoma tigrinum*) had neoplastic and non-neoplastic lesions with a peak frequency of 53%. Chemical carcinogens, especially perylene, were suspected of causing the lesions.

Papillomas occur on many species of fish and are associated with pollution and tumorigenic viruses. Other fish tumors have a genetic origin. All of these factors were considered possible causes of the papillomas in fish from the Tuskegee sewage treatment plant.

Juvenile black bullheads, yellow bullheads, and channel catfish were placed in floating and sunken cages in the final oxidation pond of the Tuskegee Sewage Treatment Plant and in a control pond to determine the effects of chronic exposure to the chlorinated effluent. Feral and caged fish from the oxidation and control ponds were examined for lesions. Persistence, histology, and ultrastructure of papillomas on wild black bullheads were studied. Transmission of the papillomas to healthy black bullheads was attempted by means of injecting cell-free tumor homogenate. Water from the oxidation pond was analysed for metals using atomic absorption spectrophotometry and for organic compounds by gas chromatography. Concentrates of the oxidation pond water were tested for mutagenicity using the Ames test.<sup>2</sup>

## Results and Conclusions

The results and conclusions from this study are:

1. Water analyses did not reveal presence of any chemical in sufficient concentration to be cited as the cause of papillomas on black bullheads in the oxidation pond. The possibility of temporal changes in composition of the waters, however, was not adequately considered during this study.
2. The acidic organic solvent extract of the oxidation pond water was mutagenic to *Salmonella typhimurium* strains TA98 and TA100 when Aroclor-induced rat liver enzymes (S-9) were included in the agar but was not mutagenic without S-9. The basic organic solvent extract was not mutagenic with or without S-9.
3. No tumors developed on adult or juvenile fish injected with cell-free tumor homogenate. The adult fish were observed for 14 months before they were necropsied. The juveniles have been observed for 140 days and observation will continue for several more months.
4. The papillomas on adult, wild black bullheads from the oxidation pond contained cytoplasmic inclusion bodies, usually in cells near the tumor surface, that were eosinophilic, PAS positive, and weakly Feulgen positive. No evidence for the presence of a virus was found during ultrastructural examination of the papillomas. Histologically and ultrastructurally, these black bullhead papillomas were similar to papillomas in brown bullheads and eels.

5. Twenty-eight adult black bullheads were taken from the oxidation pond, placed in clean water in the control pond, and observed for papilloma persistence. All 28 fish retained the lesions, even the six that survived for more than a month. Persistence of the lesions after removal of the fish from the oxidation pond indicates that the lesions are neoplastic rather than hyperplastic.
  6. During 168 days in the oxidation pond, 91% of the caged black bullheads developed focal, oral hyperplasia and stomatitis in the same mouth location as the papillomas observed in wild black bullheads from this pond. Because no pathogens were consistently associated with these lesions, the lesions in caged fish may be related to chemicals in the water. The hyperplastic lesions healed in most fish during additional exposure. Mucosal hyperplasia also occurred in other test species and in all control species, but at a maximum frequency of 14%. A similar incidence of hyperplastic lesions in fish confined to floating or sunken cages indicated that contact with the sediment or ingestion of benthic food organisms did not affect pathogenesis of the lesions. Papillomas did not develop in caged fish kept in the oxidation pond. Whether additional exposure time will lead to development of papillomas can be determined only by continuing this experiment.
2. Test oxidation pond water for mutagenicity using the Ames test. Seasonal variation in mutagenicity should be determined. Additional fractionation of the water before testing could indicate the chemical nature of mutagens present in the water.
  3. Enzyme induction in caged fish should be determined, compared to results of other parts of this study, and used as an indication of the presence of a chemical carcinogen in the water.
  4. Black bullheads injected with cell-free tumor homogenate should continue to be observed for development of tumors.
  5. Fish placed in cages during this study should be maintained in cages to determine if tumors develop after longer exposure. Additional caged fish should be placed in the oxidation pond at different times of the year to determine if the etiological agent and development of hyperplastic lesions is seasonal.
  6. Histological and ultrastructural studies of caged fish should be continued to document tissue changes in fish confined to oxidation pond water.

## Recommendations

1. Analyse water from the final oxidation pond for carcinogens. Future investigations should include collection of water samples taken during different times of the year to determine temporal changes in composition of the water.

## References

1. Grizzle, J. M., T. E. Schwedler, and A. L. Scott. 1981. Papillomas of black bullheads, *Ictalurus melas* (Rafinesque), living in a chlorinated sewage pond. *Journal of Fish Diseases*, 4:345-351.
2. Ames, B. N., J. McCann, and E. Yamasaki. 1975. Methods for detecting carcinogens and mutagens with the *Salmonella*/mammalian-microsome mutagenicity test. *Mutation Research*, 31:347-364.

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