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GREAT LAKES NATIONAL PROGRAM OFFICE
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Stephen Galarneau, Director
Office of Great Waters – Great Lakes & Mississippi River
Wisconsin Department of Natural Resources
PO Box 7921
Madison, WI 53707-7921

Dear Mr. Galarneau:

Thank you for your April 13, 2020 request to remove the *Tainting of Fish and Wildlife Flavor* Beneficial Use Impairment (BUI) from the Lower Green Bay and Fox River Area of Concern (AOC) located in Green Bay, WI. As you know, we share your desire to restore all the Great Lakes AOCs and to formally delist them.

Based upon a review of your submittal and supporting information, the U.S. Environmental Protection Agency (EPA) approves your request to remove this BUI from the Lower Green Bay and Fox River AOC. EPA will notify the International Joint Commission (IJC) of this significant positive environmental change at this AOC.

We congratulate you and your staff as well as the many federal, state and local partners who have been instrumental in achieving this environmental improvement. Removal of this BUI will benefit not only the people who live and work in the AOC, but all residents of Wisconsin and the Great Lakes basin as well.

We look forward to the continuation of this important and productive relationship with your agency as we work together to delist this AOC in the years to come. If you have any further questions, please contact me at (312) 353-8320 or your staff can contact Leah Medley at (312) 886-1307.

Sincerely,

CHRISTOPHER KORLESKI

Digitally signed by
CHRISTOPHER KORLESKI
Date: 2020.04.28 14:23:20
-05'00'

Chris Korleski, Director
Great Lakes National Program Office

cc: Briana Kupsy, WDNR
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April 13, 2020

Mr. Chris Korleski, Director
Great Lakes National Program Office
U.S. Environmental Protection Agency, Region 5
77 West Jackson Boulevard
Chicago IL 60604

Subject: Removal of the suspected Tainting of Fish and Wildlife Flavor Beneficial Use Impairment in the Lower Green Bay & Fox River Area of Concern

Dear Mr. Korleski:

I am writing to request the U.S. Environmental Protection Agency (U.S. EPA) Great Lakes National Program Office's (GLNPO's) concurrence with the removal of the suspected Tainting of Fish and Wildlife Flavor Beneficial Use Impairment (BUI) in the Lower Green Bay & Fox River Area of Concern.

The Wisconsin Department of Natural Resources (WDNR) has assessed the status of the suspected Tainting of Fish and Wildlife Flavor BUI relative to the rationale for its "suspected" designation, as no delisting target was established in 2008. Additionally, WDNR presents the results of an angler survey, natural resource and health official inquiries, and stakeholder support in the official removal of this suspected BUI. WDNR conducted a public review of the recommendation, including consultation with AOC stakeholders and technical experts in a 30-day public comment period. Comments received during the public review have been addressed.

Please find enclosed the Tainting of Fish and Wildlife Flavor Beneficial Use Impairment Removal Recommendation document, prepared by WDNR, which provides documentation to support this recommendation.

We value our continuing partnership in the AOC Program and look forward to working closely with GLNPO in the removal of BUIs and the delisting of Wisconsin's AOCs.

If you need additional information, please contact Brie Kupsky, WDNR, 920-662-5465 or you may contact me.

Sincerely,

Stephen Galarneau, Director
Office of Great Waters - *Great Lakes & Mississippi River*
Wisconsin Department of Natural Resources
608-266-1956
stephen.galarneau@wisconsin.gov

Enclosures

cc: Sue Virgilio, U.S. EPA
Leah Medley, U.S. EPA
Amy Pelka, U.S. EPA
Marc Tuchman, U.S. EPA
Kendra Axness, Wisconsin DNR
Rebecca Fedak, Wisconsin DNR
Brie Kupsky, Wisconsin DNR

Lower Green Bay & Fox River Area of Concern
Beneficial Use Impairment Removal Recommendation:
Tainting of Fish and Wildlife Flavor



Submitted to:

U.S. Environmental Protection Agency Great Lakes National Program
Office
77 W. Jackson Blvd.
Chicago, IL 60604

By:

Wisconsin Department of Natural Resources
April 2020

Acknowledgements

Prepared by:

Brie Kupsy, Lower Green Bay & Fox River AOC Coordinator
Office of Great Waters
Wisconsin Department of Natural Resources

The Wisconsin Department of Natural Resources would like to acknowledge the support provided by the Lower Green Bay & Fox River Area of Concern (AOC) stakeholders in the development of the Tainting of Fish and Wildlife Flavor Beneficial Use Impairment Removal Recommendation Document. Your local input and associated efforts were an invaluable part of the process to remove the AOC's first beneficial use impairment and reflects the incredible ongoing efforts that will enable us to continue forging the path to delisting.

Photo Credit: Christopher Rand

Disclaimer

The Great Lakes Water Quality Agreement (GLWQA) is a non-regulatory agreement between the United States and Canada, and criteria developed under its auspices are non-regulatory.

Executive Summary

In 1987, the lower Green Bay and Fox River was designated as an AOC under the GLWQA, encompassing the last 7 miles of the lower Fox River and 21 mi² of the lower bay of Green Bay. The AOC designation was primarily due to the existence of polychlorinated biphenyls (PCBs) present in sediment and water quality issues stemming from a heavily industrialized Fox River and nutrient inputs from the watershed, resulting in an aquatic environment toxic to human, fish, and wildlife health.

In 1993, eleven confirmed and two suspected beneficial use impairments (BUIs) were identified in the AOC in the Remedial Action Plan (RAP) Update. Tainting of Fish and Wildlife Flavor was listed as one of two suspected BUIs because taste and odor complaints of fish harvested from the Lower Fox River (below the De Pere Dam) were reported to the Wisconsin Department of Natural Resources (WDNR). No anecdotal or published sources found suggested that wildlife harvested within the AOC boundaries had tainted flavor or odor issues, therefore this document focuses solely on the tainting of fish flavor and/or odor.

Various federal and state water quality policies, local initiatives, and alterations to pulp and paper-making processes were implemented in the 1970-90s to ensure that water quality standards for flavor tainting substances were met. Following these reductions in point source pollution to AOC waters, the WDNR developed a direct assessment of this BUI to resolve whether the flavor tainting impairment could be confirmed to be present or if removal of the BUI could move forward. The results of the 2013 survey indicated that over 70% of anglers rated fish flavor and smell as “good” or “excellent”, and no “poor” flavor or taste ratings were given to any of the fish types/categories harvested from the AOC.

The results of this assessment were presented to AOC stakeholders in 2017 and they concurred with WDNR that no additional assessments or management actions be established and instead, a removal recommendation be developed. WDNR discussed the recommended strategy with the U.S. Environmental Protection Agency’s Great Lakes National Program Office (GLNPO), including senior managers who agreed to this approach. WDNR also inquired about complaints regarding flavor tainting in fish and/or wildlife to various local agencies and professionals who were likely to have received such complaints, none of which reported receiving any complaints or finding historical complaint documentations.

Given the water quality improvements, results from a survey assessment, local stakeholder recommendations, and professional inquiry, this BUI removal recommendation is submitted by the WDNR Office of Great Waters (OGW) and supported by AOC stakeholders.

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Purpose

The purpose of this document is to provide lines of evidence and supporting documentation associated with the removal of the suspected Tainting of Fish and Wildlife Flavor BUI in the AOC. The 1993 RAP Update considered this BUI “suspected” in the AOC, and BUI criteria were not developed in the 2009 delisting targets due to a lack of sufficient information supporting the formal listing of this BUI (WDNR, 2009). Therefore, this document delivers a list of remedial actions conducted in the AOC to-date that have likely contributed to the opportunity to remove this impairment, the results of a 2013 angler survey that largely suggests the lack of flavor and odor tainted fish harvested within the AOC, and supplemental considerations supporting removal of this BUI.

Background

In 1987, the lower Green Bay and Fox River was designated as an AOC under the GLWQA due to pollution discharges from municipal and industrial wastewater, as well as nutrient inputs from various industrial and municipal point and agricultural nonpoint sources. The sources originate and/or are transported to the AOC immediately below the De Pere Dam to the mouth of the river and extends into the head of the lower bay of Green Bay to an imaginary line drawn between Long Tail Point and Point au Sable (Figure 1). The 1993 Remedial Action Plan Update (WDNR, 1993) identified the following thirteen BUIs in the AOC, eleven of which are confirmed and two that have remained designated as suspected impairments:

Confirmed

- Restrictions on Fish and Wildlife Consumption
- Degradation of Fish and Wildlife Populations
- Bird or Animal Deformities or Reproductive Problems
- Degradation of Benthos
- Restrictions on Dredging Activities
- Eutrophication or Undesirable Algae
- Restrictions on Drinking Water, or Taste and Odor Problems
- Beach Closings
- Degradation of Aesthetics
- Degradation of Phytoplankton and Zooplankton Populations
- Loss of Fish and Wildlife Habitat

Suspected

- Tainting of Fish and Wildlife Flavor
- Fish Tumors or Other Deformities



Figure 1. The Lower Green Bay & Fox River Area of Concern

This document focuses solely on the suspected Tainting of Fish and Wildlife Flavor BUI. It should be noted that this document does not seek to address the removal of the Restrictions on Fish and Wildlife Consumption BUI or any fish consumption advisories currently in place. Fish consumption advisories are due to bioaccumulation of toxic environmental contaminants still present in the fish and wildlife in the AOC and will remain irrespective of fish flavor. There are no fish consumption advisories established in the state of Wisconsin for flavor or odor tainting. More information on current fish consumption advisories can be found on the WDNR's website at <http://dnr.wi.gov/topic/Fishing/consumption/>.

Rationale for Suspected BUI Listing Designation

Much of the point source pollution that was generated within the AOC stemmed from what was said to be one of the highest concentrations of paper mills in the world, located on the Fox River below Lake Winnebago. These mills historically released several environmental contaminants into the river which were then transported to the lower bay of Green Bay through uncontrolled effluents (Sullivan & Delfino, 1982). Specially relevant to fish flavor and odor issues was the release of several sulfur-containing compounds such as phenols, resins, and fatty acids in paper and pulp-making effluents prior to state and federal regulations of discharges, compounds that were later understood to cause organoleptic (taste and odor) effects on water and aquatic organisms (Sullivan & Delfino, 1982; WDNR *et al.*, 1983; USEPA, 1986).

While the effect of these compounds on the taste and odor of fish harvested from the Lower Fox River was not initially documented in the 1988 RAP, the 1993 RAP Update identified Tainting of Fish and Wildlife Flavor as a suspected BUI, as neither of the two International Joint Commission (IJC) listing criteria were known in sufficient detail to consider confirming the BUI:

IJC Listing Criteria: When ambient water quality standards, objectives, or guidelines for the anthropogenic substance(s) known to cause tainting are being exceeded or survey results have identified tainting of fish or wildlife flavor.

WDNR received some complaints from local anglers regarding taste and odor problems in the fish harvested in the AOC, prompting the "suspected" designation. Though the WDNR does not formally track complaints regarding fish flavor and/or odor, anecdotal reports from anglers indicated that flavor and/or odor tainting in Walleye harvested in the Fox River below the De Pere Dam was fairly common, particularly in the larger, long-lived fish. Articles published in 1983 and 1984 referred to the questionable quality of Walleye in the Fox River and provided a method of preparation for larger fish to remove the sulfite odor or "off" flavor (Figure 2), suggesting that this was a common complaint of area anglers.

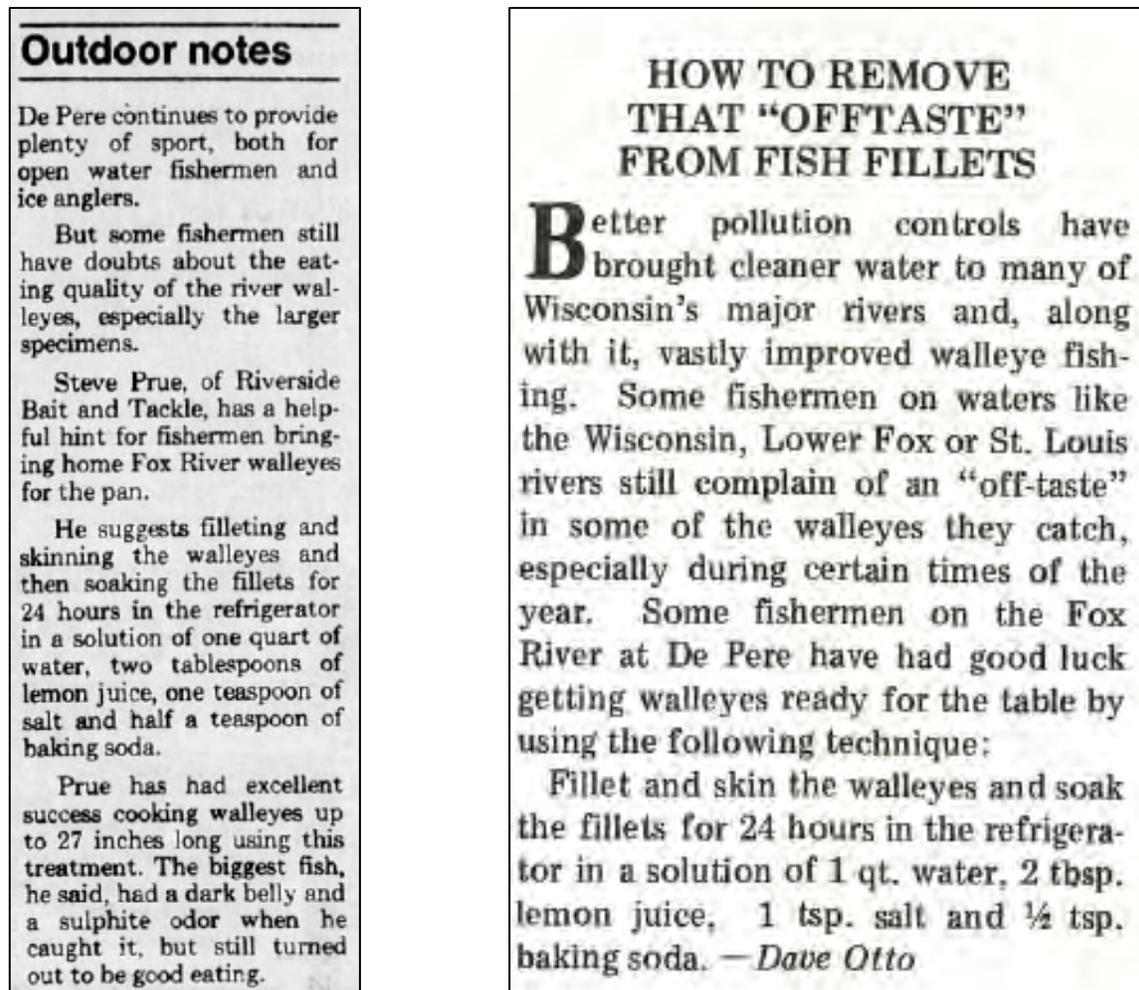


Figure 2. The article on the left was published in the Green Bay Press Gazette on February 2, 1983; the article on the right was obtained from the May/June 1984 issue of Wisconsin Sportsman.

Additionally, an article published in the *Wisconsin Natural Resources* magazine in 1990 described fish tainted with "off" flavors and odors occurring in Lake Mendota and the Wisconsin River, citing poor water quality as the main culprit though the Fox River and lower bay of Green Bay were not included (Appendix D).

Potential Sources of Compounds Producing Flavor or Odor Tainting Effects on Fish in the AOC

Three broad categories of pulp and paper making processes have historically occurred within the lower Fox River basin, all of which have contributed to some level of environmental degradation within the AOC through waste effluents discharged to the Fox River (Sullivan & Delfino, 1982). "Nonintegrated" mills purchase wood pulp or other fiber sources to manufacture paper products; "Secondary Fiber" mills utilize recycled paper products to produce a pulp that

will produce different paper products; and finally, “Integrated” mills produce their own pulp from raw wood materials to manufacture paper products (Andelin *et al.*, 1989).

The various integrated pulp-manufacturing processes (i.e. sulfite, Kraft, and semi-chemical) may have been responsible for the taste and odor problems experienced in fish caught within the AOC, as these mills discharged large amounts of spent sulfite waste to the lower reaches of the Fox River. A paper presented in the *Journal of the Water Pollution Control Federation* described the magnitude of the pulp manufacturing waste stream issue in the Fox River by stating, “in most pulping processes, roughly 50% of the original wood is converted into a salable product and the remaining 50% is waste which must be treated,” (Faulkender *et al.*, 1968). In 1968, a WDNR report identified 33 industrial and municipal waste sources on the Fox River from Lake Winnebago to the mouth of Green Bay to catalog relative waste and biological oxygen demand (BOD) discharge (WDNR, 1968). Of the 18 pulp and paper mill sources, seven were associated with the integrated pulp and paper manufacturing method, three of which were located directly within the AOC boundaries (Table 1).

Table 1. Inventory of paper mills located on the lower 40 miles of the Fox River and relative daily effluent discharge from 1966-1967. BOD = Biological Oxygen Demand; S.S. = Suspended Solids. Table obtained and expanded from WDNR, 1968.

River Miles	Source	Mill Category	Daily Effluent Discharge (gal)	Daily BOD Discharge (tons)	Daily S.S. Discharge (tons)
40.1	Kimberly Clark-Neenah Division	Nonintegrated	530,000	300	0.46
39.9	Kimberly Clark-Badger Globe	Secondary Fiber	530,000	140	.18
39.8	Gilbert Paper Co.	Nonintegrated	890,000	740	0.32
39.8	John Strange Paper Co.	Secondary Fiber	1,600,000	1,840	1.57
39.8	Bergstrom Paper Co.	Secondary Fiber	4,200,000	19,700	21.41
39.2	Kimberly Clark- Lakeview	Nonintegrated	5,250,000	1,460	3.38
38.7	George A. Whiting Paper Co.	Nonintegrated	320,000	380	0.75
33.3	Riverside Paper Corp.	Secondary Fiber	2,530,000	1,500	3.91
32.1	Consolidated Papers Inc.	Integrated Sulfite	8,130,000	30,880	4.13
29.0	Kimberly Clark- Kimberly	Integrated Sulfite	11,490,000	28,600	26.09
27.0	Combined Paper Mills Inc.	Integrated Chemi-Mechanical	3,050,000	5,760	5.53
23.0	Thilmany Pulp & Paper Co.	Integrated Kraft	26,160,000	33,660	13.98
7.0	Nicolet Paper Co.	Nonintegrated	1,620,000	580	0.98
6.8	U.S. Paper Mills Corp.	Secondary Fiber	620,000	4,060	4.29
3.7	Fort Howard Paper Co.	Secondary Fiber	11,400,000	32,720	13.94
1.4	American Can Co.	Integrated Sulfite	16,300,000	43,180	7.23
1.0	Charmin Paper Products	Integrated Sulfite	15,380,000	45,520	14.07
0.8	Green Bay Packaging Inc.	Integrated Semi-Chemical Sulfite	2,830,000	25,270	2.97

Studies Investigating Flavor and Odor Tainting Effects on Aquatic Organisms

A study conducted in the 1930s at the University of Wisconsin-Madison was one of the first to examine the effects of Kraft and sulfite mill effluents (Cole, 1935). While the dilutions employed in this study were admittedly well-above an effluent concentration level expected to be found in receiving waters (except perhaps within the immediate proximity of an industrial outfall for the highest dilution), this was one of the first studies to suggest that discharge of sulfite and Kraft effluents could potentially result in toxic, chronic, and/or sublethal effects on aquatic organisms in the presence of dissolved oxygen levels sufficient to sustain aquatic life. Several other studies undertaken between the 1930s and 1960s identified flavor and odor tainting in fish harvested from industrialized rivers and suggested this was due to a combination of compounds discharged in pulp mill effluents as well as the decomposition of discharged pulp mill fibers (Westfall and Ellis, 1938; Baldwin *et al.*, 1961; Swabey, 1965; German, 1968).

While controlled studies conducted in the 1960-1970s provided a more focused look at the effect of specific compounds on flavor tainting in aquatic organisms, very few were able to identify the actual capacity of specific compounds to impart flavor-tainting in aquatic organisms (Shumway & Palensky, 1973). In response, USEPA sponsored a three-year laboratory study that examined the influence of individual organic compounds on fish flavor in 1973 that successfully identified estimated threshold concentrations (ETCs) for 22 specific organic compounds and eventually established recommended national water quality criteria for flavor and odor-tainting effects for 24 compounds in Section 304(a) of the Clean Water Act (USEPA, 1986). This water quality criteria became regulated under NR 102.14, Wisconsin Administrative (Wisc. Adm.) Code in 1989.

In 1986, researchers from UW-Madison's Department of Food Science and Environmental Toxicology Program were contracted by WDNR to draft a document summarizing the causes of sportfish flavor and odor tainting in the Wisconsin River, and management recommendations for controlling the compounds causing the tainting. Heil and Lindsay (1986) described three distinct types of flavor tainting compounds encountered in fish collected from the Wisconsin River:

1. Alkyl-phenols: A kerosene or medicinal-like flavor in fish caused by resin acids originating from both natural (leaves, wood) and industrial sources (bark, wood fiber, wood chips).
2. Thiophenols: A sulfurous or meat-like flavor in fish caused by thiophenol and thiocresol originating from industrial paper process activities.
3. Geosmin or methylisoborneol: An earthy or musty flavor in fish caused by geosmin, methylisoborneol, or a combination of the two and originating from blue-green algae blooms or other bacteria encountered in stagnant waters.

In addition to identifying specific compounds in the Wisconsin River causing flavor and/or odor tainting in sport fish, Heil and Lindsay (1986) also identified specific periods of the year in which the flavor and odor tainting were more or less pronounced:

1. Spring (March – May): Worst period for encountering tainted fish in the Wisconsin River as effluents were not restricted during this part of the year and ice cover containing the

discharged alkyl- and thiophenols were said to create reductive biological conditions. Cold temperatures were also suggested to depress the metabolism of fish resulting in a slowed rate of fish clearing these compounds from tissues.

2. Early summer (May to July): Incidences of flavor tainting begin to subside as water warms.
3. Late summer to early fall (July to October): Some reports of flavor tainting during this period and said to be a result of increasing algal biomass and therefore increased geosmin and/or methylisoborneol byproduct production, and less of a result of alkyl- or thiophenol production as effluents were restricted during late summer low flow periods.
4. Late fall to early winter (October – January): Few reports of flavor tainting as conditions less suitable and less fishing pressure is encountered.

In general, Heil and Lindsay (1986) reported that encounters with tainted fish in the Wisconsin River were often experienced in early spring from 1977-1980, though from 1981-1986 the quality of fish considerably improved with the reduction of effluents discharged into the river as water quality improvements were made through increased state and federal regulations. A summary of federal, state, and local policy, statutes, rules and initiatives resulting in improved water quality in the AOC immediately follows.

Summary of Actions, Supplemental Lines of Evidence, and Stakeholder Recommendations Related to the Tainting of Fish and Wildlife Flavor BUI

Water Quality Improvements

While no removal targets or management actions were established for this BUI because it was not affirmatively established as an impairment, several improvements have been made in the AOC through various federal, state, and local policy, statutes, rules and initiatives that are believed to have directly and indirectly addressed this BUI:

1. Federal Policies, Rules and Initiatives
 - a. Clean Water Act (Kraft, 2015)
 - i. 1972 Amendments
 1. Established the basic structure for regulation pollutant discharges into the waters of the United States.
 2. Gave USEPA the authority to implement pollution control programs (e.g. NPDES Permit Program).
 3. Maintained existing requirements to set water quality standards for all contaminants in surface waters.

4. Made it unlawful for any person to discharge any pollutant from a point source into navigable waters, unless a permit was obtained under its provisions.
 - ii. 1977 Amendments
 1. Extended authority to include conventional, non-conventional, and toxic pollutants.
 2. Bolstered authority to eliminate toxic pollutants.
 - iii. 1986 Quality Criteria for Water, “*Gold Book*”
 1. Established recommended water quality criteria for organoleptic effects pursuant to Section 304(a) of the Clean Water Act.
 - iv. 1987 Amendments
 1. Authorized the implementation of a state revolving loan program to help local governments build wastewater treatment facilities.
 - v. 1998 “Cluster Rule” revised Effluent Guidelines and Standards for Pulp, Paper and Paperboard category (40 CFR Part 430)
 1. Established effluent limits for toxic pollutants.
 2. Published a *Technical Support Document for Best Management Practices* that reduce release of toxic, conventional, and nonconventional pollutants to surface water.
 - b. GLWQA – binational agreement between the United States and Canada that established additional goals and objectives for improving water quality in the Great Lakes.
 - i. Under the 1987 GLWQA, the U.S. and Canada established 43 discrete geographic locations as AOCs to prioritize remedial efforts within the Great Lakes.
 - ii. IJC established general listing and delisting guidelines for 14 BUIs to serve as an initial reference point for defining and addressing specific issues in AOCs.
2. State Rules and Initiatives
 - a. Natural Resources (NR) 102.14, Wis. Adm. Code established in 1989 regulating threshold concentrations for various substances causing objectionable tastes or odors in waters and aquatic organisms.
 - b. WDNR worked with the paper industry between 1985 and 1990 to identify and reduce discharges of taste and odor compounds into surface waters (see Appendix D)
3. Local Initiatives
 - a. By 1987, various industries and municipalities along the lower Fox River invested approximately \$300 million in technological pollution controls under the Clean Water Act (Mazmanian & Kraft, 2009) that have resulted in significantly less point and nonpoint source pollution in the AOC.
 - b. *Lower Green Bay & Fox River AOC RAP* and *RAP Update* established in 1988 and 1993, respectively.

WDNR 2013 Angler Survey

Given the significant water quality improvements in the AOC that have largely removed the substances (e.g. phenols, fatty and resin acids) responsible for negative impacts to fish flavor or odor, the WDNR developed a direct assessment of this BUI through the creation of an angler survey. The need for this survey was first documented in the 2012 RAP Update (WDNR, 2012), and was intended to identify whether the beneficial use was truly impaired, as a determination was needed to either establish management actions associated with this BUI or to move forward with removing it from the established list of BUIs.

Methodology

The survey was collaboratively developed by WDNR OGW, formerly Office of Great Lakes (OGL), and Science Services staff with input from various technical groups to examine responses from local consumptive users of this resource (survey located in Appendix A). The primary method of survey distribution was through the annual WDNR Creel Survey, in which anglers were supplied with paper survey forms and pre-addressed, postage-paid envelopes from March to November of 2013. Additional hard-copy surveys were distributed at the Tall Ship Festival in Green Bay in August, and electronic surveys were distributed via the Green Bay Area Great Lakes Sport Fishermen.

All completed surveys were reviewed for completeness and entered into the WDNR Surface Water Integrated Monitoring System (SWIMS) database.

Results

All Respondents

WDNR received a total of 56 completed surveys out of the ~280 surveys distributed. The average age of all respondents was ~ 50 years old, with an average of ~13 total years fishing, and an average of ~24 days/year spent fishing in the AOC (Table 2).

Most responses were collected in June (54%), followed by November (27%), and finally August (20%). The number of Wisconsin residents vastly outweighed out-of-state residents (54:2), with the majority of Wisconsin residents inhabiting Brown County (61%).

Table 2. Total and annual fishing frequency within the AOC obtained from the 2013 angler survey responses.

Total Years Fishing Frequency		
Years	N	Percent
0-5	18	32.14
6-10	11	19.64
11-15	13	23.21
16+	14	25.00
Annual Fishing Frequency		
Days/Year	N	Percent
0-10	26	47.27
11-20	13	23.64
21-30	3	5.45
31+	13	23.64

Respondents Reporting Not Eating Fish Caught in AOC

Of the 56 total respondents, approximately 26 respondents reported not eating fish caught within the AOC boundaries (Question 3). As a follow up to this question, respondents were given eight selections to choose from that best described the reason for not eating fish caught in the AOC (Question 11). Much of the reasons for not eating fish reflected concerns regarding PCB or mercury contamination and anglers that practiced only catch and release, with only 9% of responses identified to be due to previously experienced poor taste or smell from fish caught within the AOC boundaries (Figure 3).

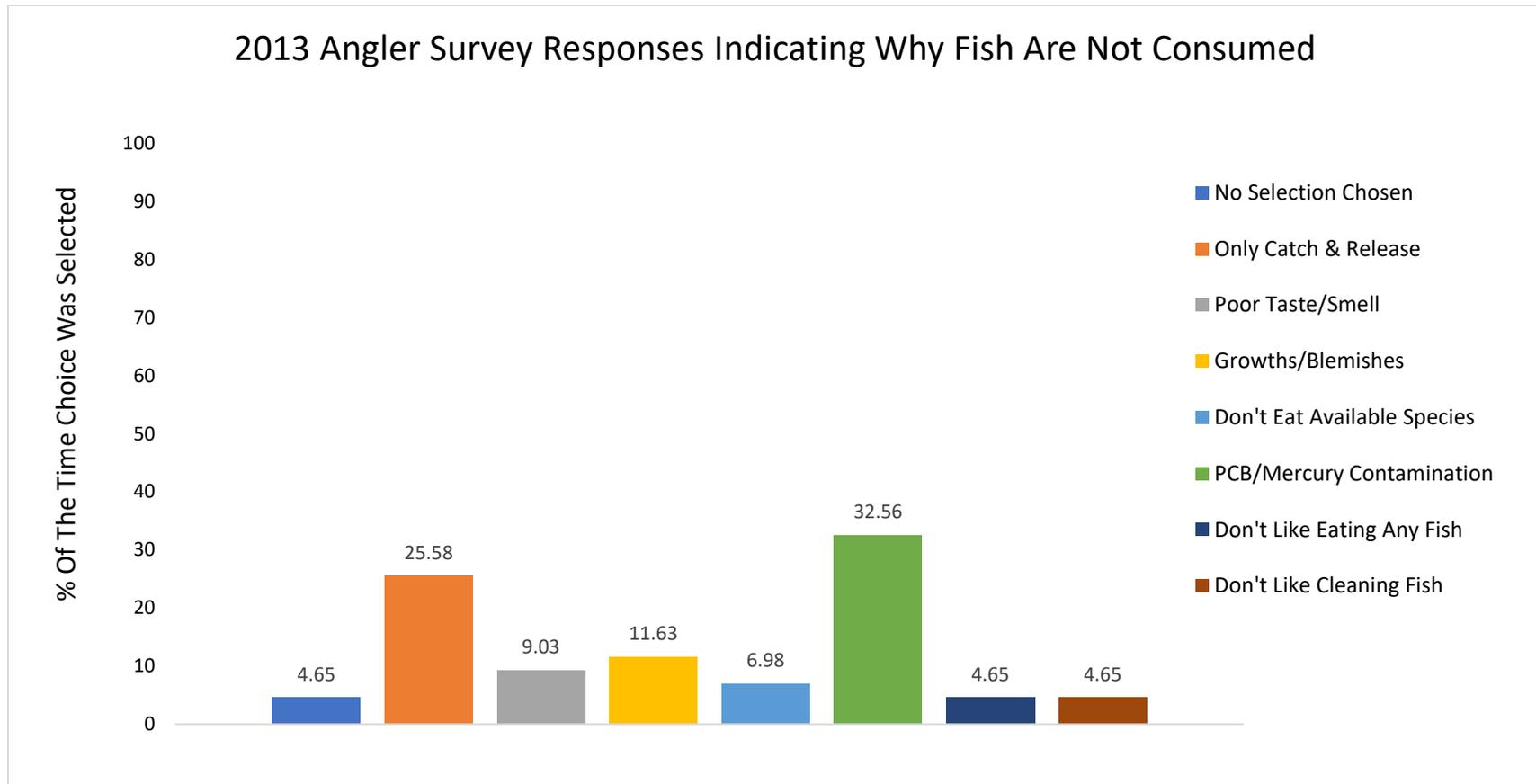


Figure 3. Results of 2013 angler survey in which anglers who indicated that they did not eat the fish caught in the survey area were given eight choices that best described the reason(s) why. A total of 26 respondents indicated that they did not eat fish caught from the survey area, with 43 different reasons indicated on the survey. The figure identifies the percentage of time each choice was indicated out of the 43 total reasons.

Respondents Reporting Eating Fish Caught in AOC

A total of 30 of the 56 total respondents indicated that they had consumed fish caught within the AOC boundaries (Question 3). Anglers were then asked to provide information on the type(s) of fish they had eaten and were asked to rate the corresponding taste and smell for each fish they consumed. Nearly all respondents reported having eaten walleye, followed by yellow perch, panfish, northern pike, bass, bottom-feeding, and other species not listed (Figure 4; Question 4). None of the fish types were reported to have “poor” taste or smell. Furthermore, a “good” or “excellent” rating was given ~77% and ~74% of responses corresponding to fish taste and smell, respectively (Table 3; Figures 5 and 6; Question 4).

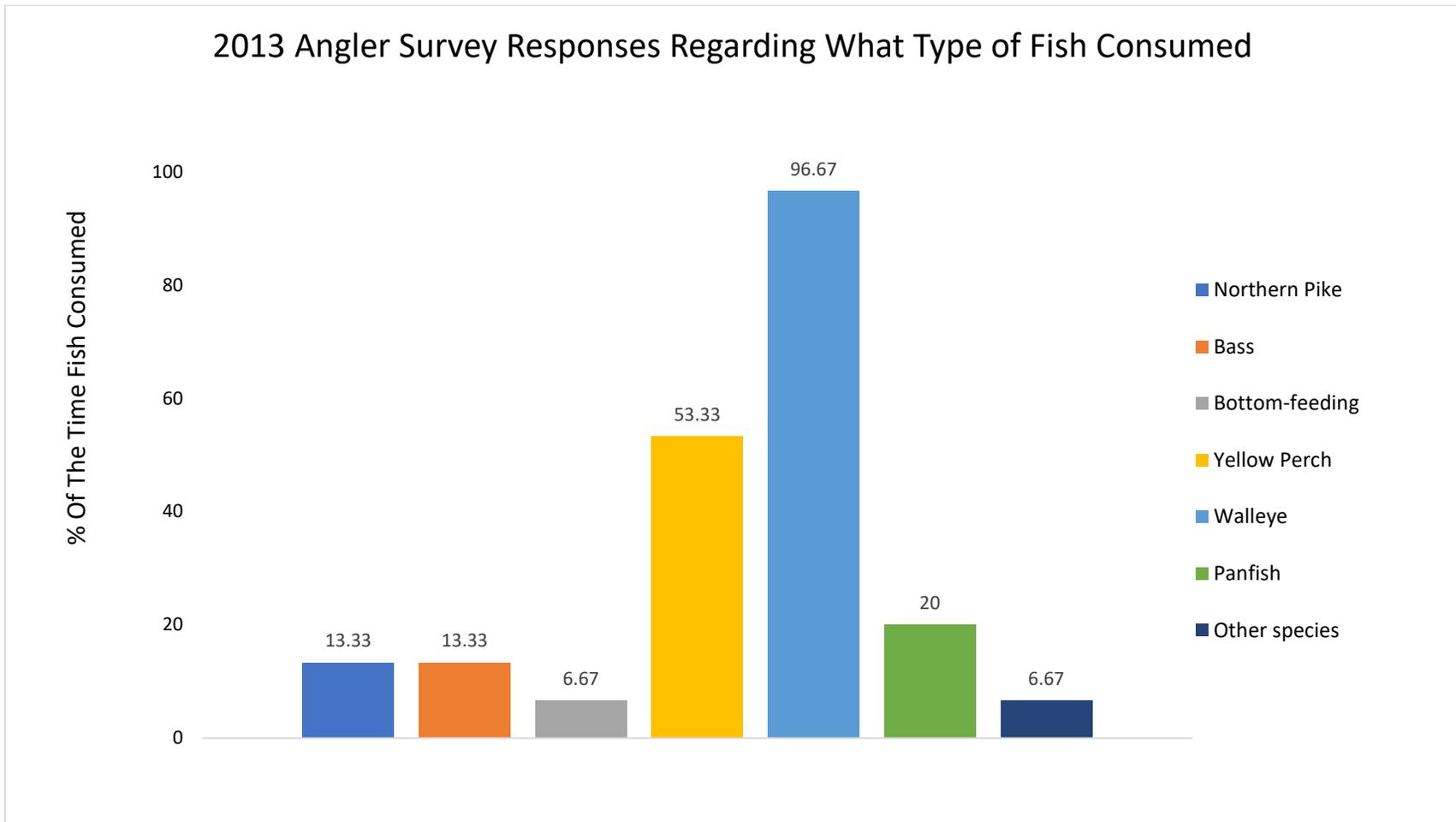


Figure 4. Results of 2013 angler survey in which anglers who indicated that they did eat fish caught in the survey area were given seven different fish types to identify as having eaten in the past. The figure identifies the percentage of time each fish type was chosen out of 63 different responses identified during survey.

Table 3. Fish flavor and smell rating frequency within the AOC obtained from the 2013 angler survey responses and the percentage of time each taste and smell rating was chosen out of 63 ratings provided in the survey.

	No Response	Poor	Fair	Good	Excellent
Taste	6.35 %	0.00 %	15.87 %	47.62 %	30.16 %
Smell	17.46 %	0.00 %	7.94 %	60.32 %	14.29 %

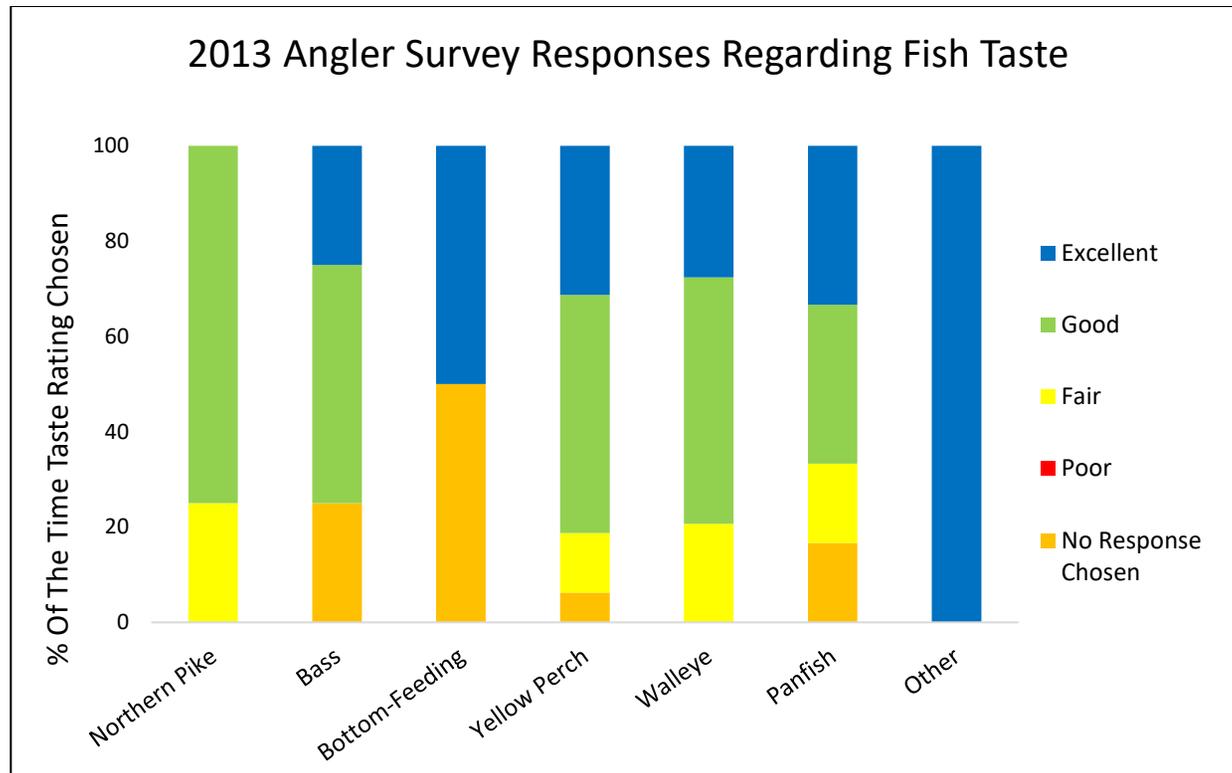


Figure 5. Results of 2013 angler survey in which anglers who indicated that they did eat fish caught in the survey area were asked to rate the taste and smell of the fish they had harvested from the AOC. The figure identifies the percentage of time each taste rating was chosen.

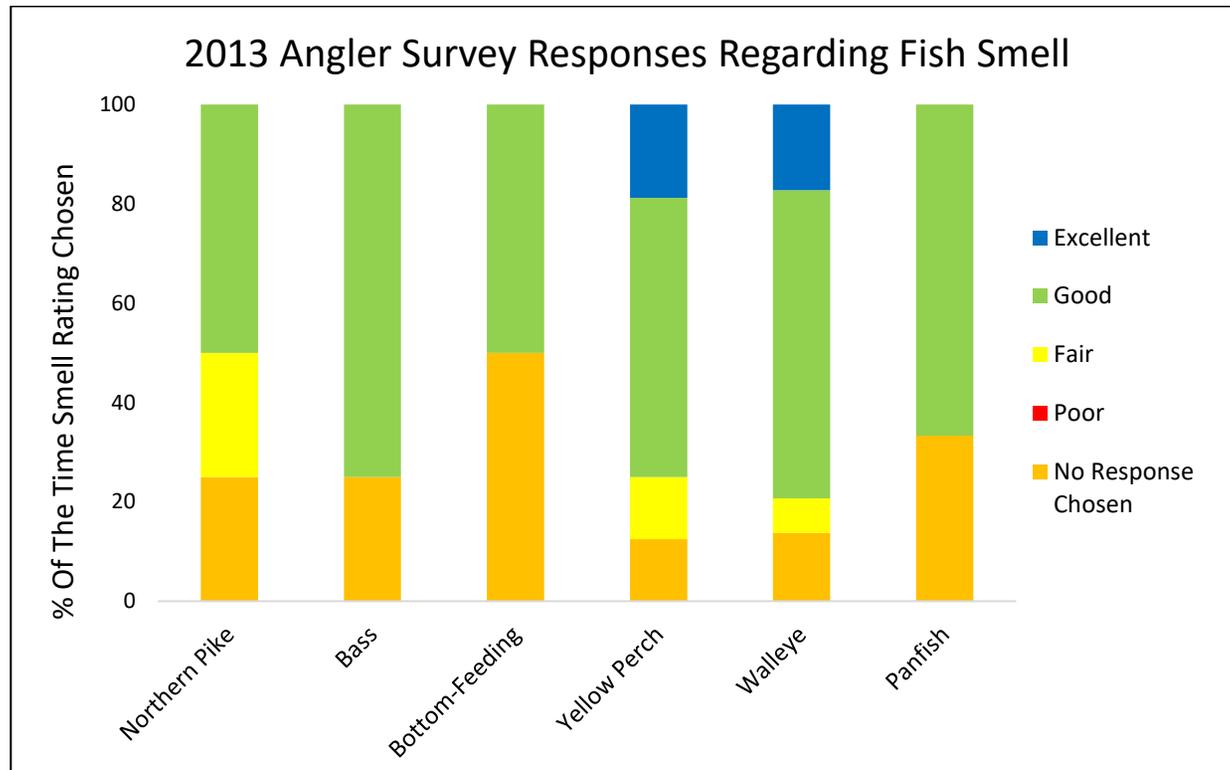


Figure 6. Results of 2013 angler survey in which anglers who indicated that they did eat fish caught in the survey area were asked to rate the taste and smell of the fish they had harvested from the AOC. The figure identifies the percentage of time each smell rating was chosen.

Only one respondent indicated that they disliked a “muddy” flavor or odor from fish harvested in the AOC, though they had not chosen poor taste or smell in any fish types surveyed (Question 5).

When surveyed on a comparison of fish taste and smell over time, 53.3% of respondents indicated no change, 23.3% indicated that fish smell and/or flavor had improved, and no respondents indicated that taste or smell was worse over time (Question 7).

Of the 24 respondents who indicated that they ate fish harvested from other areas, 12.5% indicated that fish flavor or smell were worse in fish harvested in the AOC compared to other areas, 8.3% indicated that fish flavor was better from fish harvested within the AOC, and 4.2% indicated that fish smell was better from fish harvested within the AOC (Questions 8 – 10).

Taken in whole, the results of the 2013 survey indicate that perception on the flavor and smell of fish harvested and consumed from the AOC is generally positive for anglers that consume fish, though understandable concerns remain regarding PCB/mercury contamination for anglers that indicated they do not consume fish harvested from within the AOC boundaries.

Stakeholder/Public Involvement and Recommendation

A public stakeholder meeting was held June 14, 2017 to discuss approaches for addressing the Tainting of Fish and Wildlife Flavor suspected BUI (Appendix B). Stakeholders concurred with WDNR that instead of focusing on establishing management actions or conducting further studies, that a removal recommendation be compiled with the information already available.

A 30-day public review period of this removal recommendation occurred from March 2 to 31, 2020 (Appendix C). One written (Appendix C) and one verbal comment was received in favor of the removal.

Inquiry of Natural Resource and Public Health Officials

In addition to the remedial and investigative actions presented, a further line of evidence supporting the removal of the suspected Tainting of Fish and Wildlife Flavor BUI is the lack of complaints received by the WDNR and several other local agencies in the past several years.

The following agencies were contacted via e-mail requesting information on complaints received regarding fish flavor and/or odor. None of the agencies contacted reported any complaints received within at least the last seven years (Appendix D) .

- WDNR Fisheries Biologist Steve Hogler – No complaints received during his 2010-2019 tenure at the Green Bay Service Station.
- Brown County Environmental Health and Laboratory Manager Rob Gollman – No complaints received during his 2004-present tenure.
- US Fish and Wildlife Service-Green Bay and Oneida Nation’s Water Resources Supervisor issued a joint statement that they have received no complaints of foul-tasting fish from the AOC, and no accounts of poor tasting fish have been reported to UFSWS staff working on the Bay.

Conclusion

As set forth in Annex 2 of the 1987 and Annex 1 of the 2012 Amendments of the GLWQA, the BUI addressed in this document is the “Tainting of Fish or Wildlife Flavor.” This removal recommendation outlines the rationale for listing the BUI as “suspected;” potential reasons why reports of flavor tainting in sportfish harvested from the LGBFR AOC were made in the 1960s - 1970s; federal, state, and local policy and regulations that resulted in improved water quality in the AOC; the results of a direct assessment of this BUI by the WDNR on behalf of the AOC program; and a summary of natural resource and public health official inquiries.

The WDNR and local stakeholders agree that these lines of evidence fulfill the requirements necessary to remove this suspected impairment.

Removal Statement

The WDNR AOC program staff recommend the removal of the Tainting of Fish and Wildlife Flavor BUI in the Lower Green Bay & Fox River AOC. This decision was made upon review of available data and information, a direct assessment of local anglers, inquiry of local natural resource and public health officials, recommendation of local stakeholders, and concurrence with USEPA GLNPO.

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List of Acronyms and Abbreviations

AOC	Area of Concern
BOD	Biological Oxygen Demand
BUI	Beneficial Use Impairment
CFR	Code of Federal Regulations
ETC	Estimated Threshold Concentration
GLNPO	Great Lakes National Program Office
GLWQA	Great Lakes Water Quality Agreement
IJC	International Joint Commission
LGBFR	Lower Green Bay & Fox River
NPDES	National Pollution Discharge Elimination System
NR	Natural Resources
OGL	Office of Great Lakes
OGW	Office of Great Waters
PCB	Polychlorinated biphenyl
RAP	Remedial Action Plan
S.S.	Suspended Solids
SWIMS	Surface Water Integrated Monitoring System
USEPA	United States Environmental Protection Agency
USFWS	United States Fish and Wildlife Service
WDNR	Wisconsin Department of Natural Resources
Wis. Adm.	Wisconsin Administration

Appendices

Appendix A

Lower Green Bay & Fox River AOC 2013 Angler Survey Form

Lower Green Bay and Fox River 2013 Angler Survey

The Wisconsin Department of Natural Resources (DNR) is surveying anglers to learn about their perceptions and experiences fishing in and eating fish from the Lower Green Bay and Lower Fox River, specifically the area outlined in red on the map below. This area includes the Fox River from the De Pere Dam to the mouth and the Bay out to Long Tail Point and Point au Sable. The results of this survey will be used to gather anglers' views of fish and fishing in the area and how their experiences might be improved. Note that this is not part of the annual creel survey, but is a separate survey project. Please answer the following questions so that we can understand your views and experiences. Please see the map below for the boundaries of the Survey Area.

Lower Green Bay and Fox River Survey Area



Lower Green Bay and Fox River 2013 Angler Survey

1. How many years have you fished in the Survey Area (see map)? If this is the first year, write "1." -

2. In a typical year, how many times or days do you fish in the Survey Area? If you cannot remember exactly, please provide your best estimate. _____
3. Do you ever eat fish caught from the Survey Area? YES NO

If YES, go to question 4; if NO, skip to question 11.

4. In the table below, please rate the taste and smell of each type of fish caught within the last three (3) years in the Survey Area by placing an "X" in the appropriate box. If you did not eat a particular type of fish in the last 3 years, please place an "X" in the first box (Did not eat).

Fish Type	Did not eat	Fish Taste				Fish Smell			
		Poor	Fair	Good	Excellent	Poor	Fair	Good	Excellent
Northern pike									
Bass									
Carp, bullhead, catfish, suckers									
Yellow perch									
Walleye									
Panfish (bluegill, crappie, rock bass)									
Other—please specify									

5. If you rated fish taste or smell as Poor, what tastes and/or odors have you noticed over the last three (3) years that you disliked? Please circle all that apply.

Fishy Chemical Muddy Woody Rotten

Other (describe) _____

6. Have you eaten fish caught from the Survey Area prior to the last 3 years? YES NO

If YES, go to question 7; if NO, skip to question 8.

7. If you have eaten fish caught from the Survey Area prior to the last 3 years, have you noticed a change in fish taste or smell over time?

Yes, improved Yes, got worse No, no change I don't know

If Yes, please describe in more detail (type of fish, time period, location) _____

8. Do you also eat fish from other areas? YES NO If YES, where? _____

If YES, go to question 9; if NO, skip to question 12.

9. If you also eat fish caught from other areas, have you noticed a difference in taste or smell between fish caught in the Survey Area and fish caught from other areas?

YES NO Unsure

If YES, go to question 10; if NO or Unsure, skip to question 12.

10. Please compare the taste and smell of fish caught in the Survey Area with those caught in other areas.

Survey Area Fish Taste Better Same Worse

Survey Area Fish Smell Better Same Worse

If you eat fish caught from the Survey Area, please skip to question 12.

11. If you don't eat fish caught in the Survey Area, please check all the reasons that explain why.

- I practice only catch and release.
- I've experienced poor taste or smell in the past from fish caught in the Survey Area.
- I've noticed growths or blemishes on fish from the Survey Area that cause me to not want to eat the fish.
- The species of fish that I catch in the Survey Area are those I generally don't eat.
- I have concerns about PCB or mercury contamination in fish in the Survey Area.
- I don't like the taste or smell of any fish.
- I don't like to clean fish.
- Other—please specify _____

12. Are you aware that the Wisconsin Department of Health and Department of Natural Resources jointly issue a health guide for eating fish in Wisconsin? YES NO

If YES, go to question 13; if NO, skip to question 15.

13. Are you aware of the fish consumption guidelines for the Survey Area? YES NO

If YES, go to question 14; if NO, skip to question 15.

14. How often do you follow the recommended consumption guidelines for fish caught in the Survey Area?

Always Usually Sometimes Rarely Never

15. Overall, how pleasing (beautiful) do you find the Survey Area?

Very Pleasing Somewhat Pleasing Neutral Somewhat Displeasing Very Displeasing

Please list any observations that make it pleasing or displeasing (see next page).

(15.) Please list any observations that make the Survey Area pleasing or displeasing to you.

16. Are you aware of any materials present that produce color, odor, or unsightliness to the extent that they make the area unpleasant or block your ability to access or use the water? YES NO

If Yes, please describe. _____

17. Have you noticed a change in the overall appearance of the Survey Area in the years that you have been fishing in the area?

Yes, improved Yes, got worse No, no change Don't know

If Yes, please describe. _____

18. Please add any additional comments and observations you have about fishing in the Survey Area.

19. Background information: Sex (please circle): M F Age: _____

Is Wisconsin your primary residence? YES NO

If YES, in which county do you reside? _____

If NO, in which other state or country do you reside? _____

Thank you so much for completing our survey. Please return the survey in the envelope provided to Laurel Last, WDNR, 2984 Shawano Avenue, Green Bay, WI 54313. If you have any questions about the survey, please contact Laurel at (920) 662-5103 or laurel.last@wisconsin.gov.

Appendix B

Public Meeting Notice and Agenda for Technical Stakeholder Meeting

Public meetings calendar

  All Counties ▾ 6/14/2017 for 1 Year ▾

June 2017

Wednesday, June 14

828 days old

1:00 p.m. to 3:30 p.m.      

Wednesday, June 14, 2017

Lower Fox/Green Bay Lower Fox Area of Concern Technical Stakeholders Meeting - Fish Flavor

Kress Family Library, 333 N Broadway St., **Room:** Lower level meeting room
De Pere, Wisconsin 54115 (Brown County)

 [View map of location](#)

Contact: [Megan O'Shea](#), 920-662-5465

Detailed information: Discuss approaches for addressing the [Lower Fox River/Green Bay Area of Concern](#) fish flavor Beneficial Use Impairment. Desired meeting outcome is to come to an agreement on the general approach we'd recommend for assessing the BUI.

[Agenda](#)

Published: 5/30/2017 4:07:15 PM

Retrieved from: <https://dnr.wi.gov/Calendar/Meetings/>



Lower Fox River/Green Bay Area of Concern
TECHNICAL STAKEHOLDERS' MEETING

June 14, 2017 1-3:30 p.m.

Kress Family Library

333 N Broadway St, De Pere, WI 54115

Agenda

Meeting Purpose: Discuss approaches for addressing with the Lower Fox River fish flavor BUI. Desired meeting outcome is to come to an agreement on the general approach we'd recommend for assessing the BUI.

Meeting Contact: Megan O'Shea, DNR, 920-662-5465

Topics

- **Background for impairment (Megan O'Shea)- 45 min.**

Topic goal: Megan will review the rationale for why tainted fish flavor was suspected as an impaired use for the AOC. We'll also review how other AOCs with the impairment have decided to assess it.

- **ASTM International standard practice for evaluating effects of contaminants on fish taste (Megan O'Shea)- 30 min.**

Topic goal: Megan will summarize an existing method that outlines how to assess tainted fish flavor. A copy of the method will be available at the meeting, but the method is licensed, so we will not be able to distribute copies to meeting attendees. A summary of the method will be shared at the meeting in lieu of providing a copy of the actual method.

- **Discussion about other approaches and the ASTM method (all)-45 min.**

Topic goal: Answer the question of whether the ASTM method provides a reasonable framework for BUI assessment. How might stakeholder ideas (i.e., utilizing a survey/questionnaire, holding an event for an AOC fish taste test, and using professional taste testers to determine if AOC fish have undesirable flavor) for how to address the impairment be incorporated in the assessment?

- **Identify next steps and timeframe for next meeting**

Adjourn

Last revised 05/30/2017

Appendix C

GovDelivery Announcement for Public Comment Period

From: [Wisconsin Department of Natural Resources](#)
To: [Axness, Kendra A - DNR](#)
Subject: Public invited to comment on proposal to remove Tainted Fish Flavor Impairment in Lower Green Bay & Fox River AOC
Date: Monday, March 2, 2020 2:02:50 PM

The Wisconsin Department of Natural Resources is seeking public comments on our recommendation to remove the Tainting of Fish and Wildlife Flavor Beneficial Use Impairment from the [Lower Green Bay and Fox River Area of Concern](#).

After the Lower Green Bay & Fox River was listed as an Area of Concern in 1987, the Remedial Action Plan identified Tainting of Fish and Wildlife Flavor as one of 13 environmental problems, called Beneficial Use Impairments in the AOC Program.

Tainting of Fish and Wildlife Flavor was listed as a suspected impairment at the time because taste and odor complaints of fish harvested from the Lower Fox River, below the De Pere Dam, were reported to the DNR. No anecdotal or published sources found suggested that wildlife harvested within the AOC boundaries had tainted flavor or odor issues; therefore, this document focuses solely on the tainting of fish flavor and/or odor.

Various federal and state water quality laws, local initiatives, and alterations to pulp and paper-making processes were implemented in the 1970-90s to ensure that water quality standards for flavor tainting substances were met. Following these reductions in point source pollution to AOC waters, the DNR developed a direct assessment of this BUI to resolve whether the flavor tainting impairment could be confirmed to be present or if removal of the BUI could move forward.

Water quality improvements and recent surveys of local anglers have indicated that tainted fish flavor is no longer an issue. Assessment results were presented to AOC stakeholders in 2017 and they agreed that no additional assessments or management actions be established, and instead, a removal recommendation be developed.

The removal recommendation document is available for public review and comment now until March 31, 2020, [at this link](#).

Questions and comments can be sent to:

Brie Kupsky

Lower Green Bay & Fox River and Lower Menominee River Area of Concern Coordinator

920-662-5465 or Brianna.Kupsky@wisconsin.gov

2984 Shawano Ave.

Green Bay, WI 54313

Once this impairment is removed, 12 more remain that must be addressed before this AOC can be delisted. The Lower Green Bay & Fox River is one of 43 such areas designated by the U.S. and Canada in 1987 due to the significant environmental degradation that occurred here before environmental regulations were adopted.

From: [The Hogler"s](#)
To: [Kupsky, Brianna G - DNR](#)
Subject: Re: Tainting of Fish and Wildlife Flavor BUI Removal Recommendation available for public review
Date: Monday, March 2, 2020 8:39:03 PM

Hi Brie,
I read through the delisting document for tainting of fish flavor BUI and I think it looks good.

Steve

On Friday, February 28, 2020, 09:46:12 AM CST, Kupsky, Brianna G - DNR <brianna.kupsky@wisconsin.gov> wrote:

Good morning everyone,

I'm happy to announce that the suspected "Tainting of Fish and Wildlife Flavor" beneficial use impairment removal recommendation is available on our website for public comment until March 31, 2020. This is the first of the 11 confirmed and 2 suspected impairments to be recommended for removal!

If you'd like to review the removal recommendation, it can be found on the Lower Green Bay & Fox River Area of Concern webpage located here <https://dnr.wi.gov/topic/GreatLakes/GreenBay.html>.

If you have any questions about this process or feedback on the document, please don't hesitate to call or email me at your convenience, and have a great weekend!

We are committed to service excellence.

Visit our survey at <http://dnr.wi.gov/customersurvey> to evaluate how I did.

Brie Kupsky

Lower Green Bay-Fox River and Lower Menominee River AOC Coordinator

Division of Environmental Management - Office of Great Waters
Wisconsin Department of Natural Resources
2984 Shawano Ave, Green Bay, WI 54313

(920) 662-5465 (desk)

(920) 838-5312 (cell)

Brianna.Kupsky@wisconsin.gov

"We are not limited by the past. We are committed to the future." 1988 Lower Green Bay & Fox River Remedial Action Plan

Appendix D

Natural Resource and Public Health Official Correspondence / Letters of Support

CORRESPONDENCE/MEMORANDUM**State of Wisconsin**

DATE: March 12, 2018

FILE REF: [Click [here](#) and type file ref.]

TO: Brianna Kupsky

FROM: Steve Hogler

SUBJECT: Fox River Fish Tainting Complaints

Prior to 1970, the walleye populations found in the Fox River and southern Green Bay declined sharply because of poor water quality, habitat loss and unregulated fishing. Following the enactment and enforcement of the Clean Water Act in 1972, Fish Management began an aggressive walleye restocking program to restore walleye to the Fox River and Green Bay. Walleye were stocked from 1973 through 1984 in the Fox River and southern Green Bay until stocking was halted to look for naturally reproducing walleye. Comprehensive fish surveys were conducted from 1984 through 1993 to assess the walleye population. These surveys found substantial walleye natural reproduction throughout southern Green Bay and the Fox River to extent that stocking was not resumed.

With increasing walleye populations, fishing effort and walleye harvest increased substantially throughout the 1980's continuing through the present. Some anglers in the 1980's indicated that they believed walleye flavor was tainted. Verbal and perhaps written comments were made to Fisheries staff that those walleye caught from the Fox River tasted of sulfur. Although, never documented or indicated by testing, fish flavor tainting was added to several plans including the Remedial Action Plan as an impairment.

Since the 1980's, water quality standards have been strengthened and waste water treatment plants have reduced the level of chemicals identified as causing fish flavor tainting from reaching the Fox River. Since January 2011 when I transferred to Green Bay, I have not received any written or verbal comment regarding walleye flavor caught from the Fox River or southern Green Bay.

I recommend removing Fish Flavor Tainting as a beneficial use impairment for the Fox River/Green Bay AOC.



From: Gollman, Rob K. [mailto:Gollman_RK@co.brown.wi.uw]
Sent: Monday, October 23, 2017 12:41 PM
To: Kupsky, Brianna G – DNR <Brianna.Kupsky@wisconsin.gov>
Subject: RE: Complaints Regarding Fish from Fox River / Green Bay

Hi Brie,

Our Public Health Environmental Division has not received any complaints to my knowledge regarding “off-flavors” in fish caught in the AOC. We have worked with the Wisconsin Department of Health Services in their posting of Fish Consumption Advisory signage in Brown County for quite a few years now. I have been with Brown County Public Health for over 13 years and I cannot recall *any* complaints during that time period regarding flavor of fish caught.

I hope this helps.

Best Regards,

*Rob Gollman, BS, RS
Environmental Health and Laboratory Manager
Brown County Health and Human Services Department
Public Health Division
610 S. Broadway Street
Green Bay, WI 54305
Phone: 920-448-6418
Fax: 920-448-6479*



The Brown County Health and Human Services Department-Public Health Division protects and promotes individual and community health through education, regulation, and leadership to empower community members to attain well-being across the lifespan.

From: Kupsky, Brianna G – DNR [<mailto:Brianna.Kupsky@wisconsin.gov>]
Sent: Thursday, October 19, 2017 9:27 AM
To: Gollman, Rob K.
Subject: Complaints Regarding Fish from Fox River / Green Bay

Good morning Rob,

I work for the Wisconsin DNR under the Office of Great Waters Program and am a Lake Michigan Area of Concern project assistant. Currently, I am working on drafting documentation for the removal of the Tainting of Fish and Wildlife Flavor beneficial use impairment for the Lower Green Bay and Fox River Area of Concern that will be submitted to EPA for concurrence in the near future.

One of our lines of evidence for removal of this impairment is that the Wisconsin DNR hasn't received any complaints from area anglers regarding "off-flavors" in the fish caught in the AOC in several years. However, I wanted to extend this to the Brown County and ask whether or not your department has received any complaints regarding fish flavor, or how I might go about obtaining that information? Any insight you have would be greatly appreciated!

Thanks,

We are committed to service excellence.

Visit our survey at <http://dnr.wi.gov/customersurvey> to evaluate how I did.

Brie Kupsky

Lake Michigan AOC's Water Resource Management Specialist
Division of Environmental Management - Office of Great Waters
Wisconsin Department of Natural Resources
1155 Pilgrim Rd., Plymouth, WI 53073
(920) 893-8527
Brianna.Kupsky@wisconsin.gov





United States Department of the Interior

FISH AND WILDLIFE SERVICE
MN/WI Ecological Services Office
4101 American Boulevard East, Bloomington, MN 55425
Phone (952) 252-0092 ext. 210



11/08/2018

The U.S. Fish and Wildlife has not received complaints of foul tasting fish from the Lower Green Bay/Fox River Area of Concern. Additionally, no accounts of poor tasting fish have been reported to our staff working on the Bay or to the Oneida Nation's Water Resources Supervisor James Snitgen. Tainting of Fish Flavor BUI is not related to our Agency's mission and we lack data and technical expertise to assist in evaluating.

Sincerely,

A handwritten signature in black ink, appearing to read "Peter J. Fasbender".

Peter J. Fasbender
Field Supervisor

Appendix E

Additional documents pertaining to fish flavor or odor issues in Wisconsin

1981 thesis on fish flavor tainting compounds in Wisconsin waterbodies

ISOLATION OF VOLATILE COMPOUNDS AND EVALUATION OF POTENTIAL ROLES
OF SELECTED COMPOUNDS IN THE FLAVOR-TAINING OF
UPPER WISCONSIN RIVER FISH

BY

NANCY ANN LANE

A thesis submitted in partial fulfillment of the
requirements of the degree of

MASTER-OF SCIENCE

(Food Science)

at the

UNIVERSITY OF WISCONSIN - Madison

1981

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CHAPTER 2

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INTRODUCTION

The Upper Wisconsin River has been recognized as a relatively consistent source of off-flavored fish for many years. Sportsfishermen have complained of a variety of off-flavors in cooked fish from this portion of the stream, as well as odors from freshly filleted fish in the spring of the year. As a result of water quality and tainting problems, overall usage of the sportsfishing and some portions of the river are considered restricted because of disinterest by some potential recreational users.

The study areas selected for this investigation included the headwaters, Rainbow Flowage, which has no industrialization or municipal sewage outfalls. The fish from this area could be considered free from any effects of industrial contaminants. The downstream sites included Lake Wausau and the Mosinee Flowage, which are separated from one another by several impassable dams so that fish from these two study sites could not intermingle. Several pulp and paper mills are located along the river between Rhinelander, and the lowest downstream study site. Other sources of contaminants related to man-related activities in the downstream study areas are municipal sewage outfalls and run-off from farms.

Water at the downstream sites was dark brown in color and contained small filament or fibers, and foaming occurs at certain times of the year, especially below dams.

As a part of a water quality program of the Wisconsin Department of Natural Resources, there was a desire to determine the identity

and sources of the chemical substances responsible for the off-flavors observed in the fish residing in the study area. The literature indicates that earlier studies have associated pulp and paper mills with tainting problems in fish. While these studies have established a definite relationship between this industry and some tainting problems, the causative agents and sources of these agents remained undetermined.

The specific objectives of this investigation were to systematically identify the malodorous volatile constituents contributing to the off-flavored fish obtained from the Upper Wisconsin River using methods which had previously been demonstrated to be useful for this type of work, and to attempt to determine the origin of the offending flavor compounds.

Chapter 1

LITERATURE REVIEW

REVIEW OF LITERATURE

Chemistry of untainted fish flavors

Each variety of fish has its own characteristic flavor which is a result of its unique chemical composition, and its intimate relationships within its environment. Untainted flavor characteristics, owing to individual compositional differences, arise as a result of species, sex differences, anatomical differences, physiological factors, nutritional status, seasonal and environmental conditions (Jacquot, 1961). Very little of a chemical nature is known about the cause of innate differences in the flavors of fresh fish of different species. However, worth noting are the proximate compositions of fish and shellfish as noted by Aitken and Connell (1980). Lean species contain typically 0.3-1.0% lipid, most of which is phospholipid and the remainder triglyceride. Lipid content in fatty species can be as much as 30% and vary seasonally to as low as 1%, reflecting a major fluctuation in triglyceride content. In lean, as well as fatty species, the proportion of protein is in the range of 15-18%. The remaining content of nitrogenous substances (1-3%) being made up of a multiplicity of low molecular-weight compounds.

Most fresh fish have a distinctive, but bland odor which characterizes the species in question (Stansby, 1962). This odor (aroma) cannot be attributed to a single compound or to a particular class of compounds. Rather, the odor profile of fresh untainted fish is a sum of all the sensory effects produced by a large number of volatiles of different structures present in a particular quantitative proportion

(Ohloff and Flament, 1978). In particular, the volatile flavor components are the result of bacterial, autolytic, or thermal degradation processes (or any combination of the aforementioned) which alter the chemical composition of raw fish flesh.

A series of reports (Obata et al., 1949, 1950; Obata and Yamanishi, 1952, as noted by Steinke, 1978) indicated that a variety of fish-like aromas could be simulated by selective combination of chemical compounds. Trimethylamine, methylmercaptan, piperidine, pyridine, pyrrolidone, gamma-aminovaleric acid, gamma-aminovaleraldehyde, indole, lysine, monosodium glutamate, and taurine were the compounds used in varying proportions to yield a number of distinctive fresh fish-like, fish soup-like, broiled fish-like, and dried fish-like aromas. In a paper by Ikeda (1979), analyses of the muscle constituents of fish and shellfish revealed that 95% or more of the non-protein nitrogen was accounted for by the following compounds: amino acids, imidazole dipeptides, guanidine compounds, trimethylamine oxide, urea, betaines, nucleotides, and compounds related to nucleotides. Reports (Yamato et al., 1970; Hornstein, 1967; Watanabe and Sato, 1970; Watanabe and Sato, 1971; and Harkes and Begemann, 1974, as noted by Konosu, 1979) have revealed the distribution of nitrogenous components in the muscle extracts of several species of fish. Jones (1967), as cited by Aitken and Connell (1980), reported that the array of low molecular-weight compounds is typical of any muscle and that high concentrations of certain nitrogenous compounds, namely trimethylamine oxide and urea, are of considerable significance

as odor precursors. Moncrieff (1944) and Stansby (1962) noted that methyl (mono-, di-, and tri-) amines are frequently associated with fishy odors and flavors. The methylamines are produced by microbial degradation of trimethylamine oxide. Jones (1967) noted that dimethylamine and methanol were formed from the degradation of trimethylamine oxide. Wick et al. (1967) identified ethylamine, diethylamine, n-propylamine, and butylamine in fish protein concentrate. Konosu (1979) reviewed taste-producing properties of hypoxanthine and histidine, two degradation products of nucleotides. Inosine and hypoxanthine could be important because of possible contributions to bitterness, and because they can serve as indices of the loss of flavor-enhancing nucleotides in fish. Jones (1961) reported that inosine is formed by complete dephosphorylation of inosine-5'-mono-phosphate. Jones (1961) further reported the transformation of inosine to hypoxanthine in sufficient concentrations to contribute to a bitter character of fish muscle. Geiger (1948) and Geiger et al. (1945) as cited by Steinke (1978) reported the production of histamine from the decarboxylation of free histidine with the tissue system of fish. Kimata (1961) determined that histamine concentration was dependent upon the extent of deterioration. Aitken and Connell (1980) reported that during spoilage or frozen storage, trimethylamine, urea and other precursors present in fresh fish, break down to ammonia, dimethylamine, monomethylamine, and trimethylamine, all of which may be detected in high concentrations after heating the stored fish. Jones (1961) reported that the concentration of ammonia in fish is dependent on the deamina-

tion of adenosine-5'-triphosphate prior to and immediately following death. Reay and Shewan (1949) as cited by Steinke (1978) reported the production of ammonia during refrigerated storage of fish through deamination reactions of proteolytic microorganisms. Additionally, of the volatile nitrogenous bases found in freshly caught fish, ammonia is present in the greatest concentration (Reay and Shewan, 1949).

Non-enzymatic browning, through the Strecker degradation and Amadori transformation reaction, serves as a source of odorous carbonyls. The condensation of carbohydrates with amino acids during cooking leads to the formation of heterocyclic compounds (Wasserman, 1979). Among about 3,000 known constituents of aroma (Ohloff, 1978, as cited by Ohloff and Flament, 1978), volatile heterocyclic compounds resulting from non-enzymatic browning reactions between odorless precursor carbohydrates and amino acids, could also contribute significantly. Carbonyl compounds are also readily formed by the oxidative deterioration of fish lipids. Lipids from both marine and freshwater fish are characterized by numerous long-chain, highly unsaturated aliphatic acids. Exler et al. (1975) summarized the compositional data from over 500 reported on the lipids and fatty acids in all fish species and found the predominant fatty acids to be: 14:0, 16:0, 16:1, 18:0, 18:1, 18:2, 18:3, 18:4, 20:1, 20:4, 20:5, 22:1, 22:5, and 22:6. Oxidative deterioration products of fatty acid moieties include saturated and unsaturated aldehydes, acids, ketones and alcohols. Aitken and Connell (1980) listed a large number of carbonyl compounds which have been noted in heated fish and authen-

ticated by mass spectrometry (see Table I). Several researchers have identified specific flavor compounds resulting from lipid oxidation of stored fish. c-4-Heptenal was reported by McGill et al. (1974) to be responsible for the fish meal, cardboard odor, and taste of cold stored cod. Seals and Hammond (1970) implicated c-4-heptenal as responsible for fishy flavors in rancid soybean oil. McGill et al. (1974) attributed numerous flavor responses to c-4-heptenal, including painty, wet cardboard, turnipy, green, fishy, and creamy. McGill et al. (1977) reported that hept-t,2-enal and hept-t,2,c,4-dienal were associated with fishy, cardboardy flavors caused by lipid oxidation. Swoboda and Peers (1977) reported that the fishy aroma impact of 2,4-dienals was probably due to a complex sensation of the aroma of decadienal and -trienal rather than one individual compound. The fishy character associated with stored butter has been reported (Forss et al., 1960; Pont et al., 1960; and Stark and Forss, 1962) to be caused by combinations of low molecular weight carbonyls, especially n-hexanal, n-heptanal, hex-2-enal, and oct-1-ene-3-one. Oct-1-ene-3-one has been reported to be an oxidation product of linolenic and more highly unsaturated fatty acids (Maga, 1981).

Boelens et al. (1974) reported that hydrogen sulfide and thiols are often constituents of food flavors. McLay (1967), Hughes (1964), and Wong et al. (1967) reported the presence of hydrogen sulfide in fresh seafood products. Hughes (1964) and McLay (1967) noted the production of dimethyl sulfide and methyl mercaptan during cooking of herring. Dimethyl sulfide, hydrogen sulfide and methyl mercaptan were

Table I. Carbonyl compounds in heated fish.¹

Formaldehyde	Hept- trans -2, trans -4-dienal
Acetaldehyde	Non- trans -2, trans -6-dienal
Propanal	Dec- trans -2, cis -4-dienal
Butanal	Dec- trans -2, trans -4-dienal
Isobutanal	Furfural
2-Methylbutanal	Benzaldehyde
3-Methylbutanal	o-Tolualdehyde
Isopentanal	Terephthalaldehyde
2-Methylpentanal	Phenylacetaldehyde
Hexanal	Acetone
Heptanal	Butan-2-one
Octanal	3-Hydroxybutan-2-one
Nonanal	Pentan-2-one
Decanal	Hexan-2-one
Tetradecanal	Hexan-3-one
Hexadecanal	4-Methylpentan-2-one
Octadecanal	Heptan-2-one
Propenal	Hept-3-one
But- trans -2-enal	Octan-2-one
Pent- cis -2-enal	Nonan-2-one
Hex- cis -e-enal	Nonan-5-one
Hept- trans -2-enal	Undecan-2-one
Hept- cis -4-enal	Hex-3-ene-2-one
Oct- cis -2-enal	Oct-3-ene-2-one
Oct- trans -2-enal	Oct-1-ene-3-one
Non- trans -2-enal	Oct-3,5-diene-2-one
Dec- trans -2-enal	Acetophenone
Hept- trans -2, cis -4-dienal	Cyclohexanone
5-Aminopentanal	

¹from Aitken and Connell, 1980. p. 245.

identified in boiled oysters by Yueh (1961). Thompson (1963), Hughes (1964), and Schutte (1974) reported possible precursors to be free sulfur-containing amino acids and thiamine.

A variety of other decomposition compounds has been reported in fresh fish as a result of heating. These include hydrocarbons (McGill et al., 1977; Wong et al., 1967), furan and furan-derivatives (McGill et al., 1977; Quist and von Sydow, 1974), pyrazines (McGill et al., 1977), organic acids (McGill et al., 1977), alcohols (McGill et al., 1977; Quist and von Sydow, 1974; Wong et al., 1967), and mercaptans, sulphides, disulphides, thiophenes and thiazoles (McGill et al., 1977; Quist and von Sydow, 1974; Motohiro, 1962; and Wong et al., 1967).

Mechanism of off-flavor uptake

Fish are very susceptible to absorbing chemicals from their environment or diet, which can result in objectionable off-flavors.

In freshwater fish, water passes directly through the gill membrane, not in saltwater fish. Thus, the transmission of odorants from contaminated fresh water to fish flesh is more rapid in saltwater fish where regulation by the gill membrane and digestive tract membrane occurs. Lovell and Sacky (1973) reported that catfish developed a musty, muddy taint within 48 hrs of holding in tanks with odor-producing algae. Maligalig et al. (1975) found that fish in aquaria with 15 ppm 2-pentanone or dimethyl sulfide were judged to have detectable flavor differences within 15 minutes of exposure. He also found that flavor scores paralleled odorant concentration in the tanks. The rapid transmission of odorants observed by these investi-

gators led them to conclude that transmission of odorants was directed primarily through the gill membrane. Other factor(s) which may affect or control odorant concentration in fish muscle include solubility in the blood and fish flesh constituents (Reineccius, 1979).

The influence of diet on off-flavors in fish

The influence of diet on fish flavor was demonstrated by Maligalig et al. (1973). These workers fed pond-reared catfish (Ictalurus punctatus) diets of either turkey livers or floating cereal. The turkey liver flavor notes became apparent in the fish flesh after 19 days of feeding and became more pronounced as the length of time on the diet increased. The cereal diet resulted in a cereal-like flavor note in the flesh after 33 days of feeding.

Motohiro (1962) reported an off-flavor described as being petroleum-like in canned Chum salmon (Oncorhynchus keta). The off-flavor was reported by Motohiro to be caused by the thermal degradation of dimethyl- β -propiothetin to dimethyl sulfide. The dimethyl- β -propiothetin was found to be present in a pteropod which the fish were consuming as a major part of their diet. Dimethyl sulfide, derived from dimethyl- β -propiothetin, was also found to be responsible for an off-flavor described as blackberry in cod from the Labrador coast (Sipos and Ackman, 1964; Ackman et al., 1966, 1967) and was also related to a pteropod in their diet.

Earthy, musty, muddy flavors have been associated with soil and lake and river muds (Wakesman, 1959) and were associated with actinomycetes by Berthelot and Andrea in 1981. Muddy, earthy taints

in fish were linked with actinomycetes and blue-green algae by Thaysen (1936), Thaysen and Pentlow (1936), Gerber and Lechevalier (1965) and Safferman et al. (1967). Odors from biological origins such as the actinomycetes have caused problems for water treatment operators (Rosen et al., 1970), as well as commercial fisheries, such as the tainting of fish in S.W. Manitoba lakes which damaged the commercial fishing industry (Yurkowski and Tabachek, 1974). Earthy, musty, muddy types of odors have been associated with geosmin, methyl-isoborneol, cadin-4-ene-1-ol and 2-isopropyl-3-methoxypyrazine, all have been shown to be metabolites of actinomycetes (Gerber, 1979).

The subject of earthy, muddy flavors has been extensively reviewed and discussed by Steinke (1978), thus the purpose of this discussion will be to summarize reports which have appeared since his extensive review.

Geosmin, trans-1, 10-dimethyl-trans-9-decalol, was reported to be produced by at least 17 species of actinomycetes (Gerber, 1979) and one species of blue-green algae (Gerber, 1979). Persson (1978) listed 18 species of geosmin producing species of blue-green algae. He also reported that conditions of turbidity and excess eutrophication caused by sewage and farm run-off plus favored temperatures and light conditions caused Oscillatoria agaradhii to be dominate species in a brackish, hypertrophic Finnish Bay which had a muddy odor associated with it and its fish. Persson (1978) further reported that the actinomycetes apparently have a dual role in their association with geosmin caused odor problems. They can synthesize the compound as well as

enhance the nutrients available for geosmin synthesis by liberating them from decaying algal filaments (O. agaradhi).

Steinke (1978) observed evidence of at least two 1,10-dimethyl-9-decalol enantiomers responsible for beet-like and marshy, muddy odors in a gas chromatographic analysis of extracts obtained from muddy-flavored Coho salmon (Oncorhynchus kisutch) obtained from Lake Michigan (Wisconsin) and mud and sediment samples obtained from Mud Lake (Wisconsin), a non-industrially or municipally affected lake. He found that chemically-synthesized mixtures of the three isomers of 1,10-dimethyl-9-decalol had a more fruity, camphoraceous odor than naturally obtained isomers. He further suggested that geosmin enantiomers may be present in natural systems because of the differences in odors observed in the GC effluents.

Van Gemert and Nettenbreiger (1978) reported the threshold concentration of geosmin in water to be 0.012 ppb. Steinke (1978) reported the threshold of geosmin in water and cod to be 0.02-0.05 ppb and 0.05 ppb, respectively. Yurkowski and Tabachek (1974) reported the odor threshold of geosmin in rainbow trout to be 0.06 ppb.

Geosmin has also been associated with musty undesirable off-flavors in dry beans (Buttery et al., 1976), raw beet juice (Murray et al., 1975) and ground cooked beets (Acree et al., 1976). Geosmin is frequently found associated with 2-methylisoborneol in tainted food systems. Methylisoborneol, more volatile than geosmin (Gerber, 1979), exhibits a musty, muddy odor or a camphoraceous odor depending upon not only its concentration, but also the sensitivity of the

perceiver (Persson, 1980). Van Gemert and Nettenbreijer (1978) reported the threshold of 2-methylisoborneol to be 0.02 ppb in water. Persson and York (1978) reported the threshold of this compound to be 0.18-0.020 ppb in water. Persson and York (1978) further reported the threshold of 2-methylisoborneol in pike to be 0.07 ppb and Steinke (1978) reported its threshold to be 0.02 ppb in cod. Persson and York (1978), and Steinke (1978) concluded that 2-methylisoborneol would be expected to be an extremely effective flavor causing compound in any system.

Methylisoborneol has been reported to be produced by at least one species of blue-green alga, (Lyngbya cf. cryptovaginata Schkorbatov), by Tabachek and Yurkowski (1976). It has been isolated from soil (Buttery and Garibaldi, 1976) and from odor polluted waters (Rosen et al., 1970; Piet et al., 1972; Tsuchiya, 1974). Steinke (1978) reported the occurrence of 2-methylisoborneol in Coho salmon (O. kitsutch) and in sediments from Mud Lake (Wisconsin) to be associated with copious summer algal bloom and substantial shoreline cattail (Typhy latifolia) decomposition. Persson and York (1978) demonstrated that rainbow trout (Salmo gairdneri) held in a continuous flow aquaria containing 2-methylisoborneol developed a muddy odor within two days. This finding indicated to them that the alimentary tract of the fish provided an additional route of uptake of the muddy odor compound.

Geosmin and 2-methylisoborneol were associated with muddy taints in commercial fish, including walleye (Stizostedion vitreum vitreum), northern pike (Esox lucius), lake herring (Coregonus artedii) and lake

whitefish (Coregonus clupeaformis), from an impounded lake in South-western Manitoba by Yurkowski and Tabachek (1980). They concluded that a recent impoundment and additional organic matter in the lake caused enhanced blue-green algal growth similar to that previously observed in eutrophic pot hole lakes. Muddy odor in bream (Abramis brama) was correlated with the amount of blue-green algae (O. aganadhi) which produced geosmin when grown in culture by Persson (1978). Odorous actinomycetes, also isolated from the water source from which the bream were obtained, produced geosmin and 2-methylisoborneol (Persson, 1979). Persson also noted a time lag between maximal actinomycetes concentration and off-flavor development in this system. He concluded that this could have been caused by the algal species available to the actinomycetes and the presence of competing bacteria. He concluded that the algae was the primary producer of the muddy odor compound in this instance.

Isopropylmethoxypyrazine has been identified in galbanum oil and peas (Murray et al., 1970) and potatoes (Buttery and Ling, 1973). Miller et al. (1973) isolated it from Pseudomonas perolens grown on sterile fish muscle. Buttery and Ling (1973) found it to be responsible for the musty odor in sterile milk caused by P. taetrolens (Morgan et al., 1972) and a musty, potato-like odor of spoiled, chilled fish (Miller et al., 1973). The threshold concentration for this compound was reported to be 0.002 ppb by Seifart et al. (1970). It has not yet been identified as an odor producing substance in water, but this is probably due to its exceedingly low threshold and diffi-

culty of recovering a sample of adequate size (Gerber, 1979).

The influence of environmental pollutants on off-flavors in fish

Welch et al. (1981) reported that n-alkanes (isolated from fish in major U.S. watersheds), odd-numbered alkanes (C_{13-19}), seem to be most abundant and C_{17} was the most predominate compound of the series which compose substantial background concentrations present naturally in fish. These compounds appear to be the result of normal biosynthesis in aquatic ecosystems (McInnes et al., 1980). If the balance of hydrocarbons is disturbed by absorbance of even trace quantities of unnatural hydrocarbon mixtures, objectionable flavors can result. Petroleum products are responsible for numerous reports of tainting incidents in fish. Work attempting to link off-flavors with specific hydrocarbons has succeeded in establishing that an environment polluted by oil or fuel spills can produce tainted flavors in fish flesh, however, attempts to determine the specific offending compound(s) have met with marginal success.

Rainbow trout (*S. gairdneri*) in the Bow River downstream from Calgary, Alberta, acquired an oily taste which was related to petroleum refineries (Krishnaswami and Kupchanko, 1969). They found that trout would acquire the offending taste within 24 hrs if their environment contained an equivalent of 1/100th dilution of petroleum waste water. Vale et al. (1970) and Shipton et al. (1970) reported that sea mullet (*Mugil cephalus*) taken from areas of Moreton Bay and Brisbane River, Queensland, Australia, which had docks, sewage outlets and/or heavy industry had a kerosene-like taint. Volatiles isolated

from the tainted mullet were found to resemble kerosene hydrocarbons which suggested that hydrocarbons from polluted waters were responsible for the tainting problem. Connell (1978) reported results which indicated that the hydrocarbons absorbed by the sea mullet are deposited into different segments of muscle tissue in a manner proportional to the lipid content of the muscle tissue. Metabolic processes of the sea mullet resulted in a preferential degradation of n-alkane hydrocarbons and an enrichment of isoalkane and related compounds.

Mackie et al. (1972) reported that hydrocarbons present in fuel oil-like flavored brown trout (Salmo trutta L.) caught 11 days after a diesel fuel oil spill occurred in their environment were identical to those found in diesel fuel. Ogata and Miyake (1973) concluded that toluene from nearby refineries imparted offensive odors to fish and eels captured near Mizushima, Japan. They also reported that xylenes, benzene, 1-hexene, thiophene, olefins in gasoline and some aliphatic hydrocarbons could cause taints in fish. Ogata et al. (1977) exposed eels to suspensions of crude oil in water under controlled laboratory conditions. They reported that paraffins (C_{13} - C_{15}), organic sulfur compounds and aromatic hydrocarbons accumulated in the fish flesh and resulted in the fish flesh having an obnoxious odor. Ogata and Ogura (1976) reared green fish (Girella punctata) and eels (Anguilla rostrata) in two types of artificially oil polluted waters, one with untreated mineral oil and the other with residual chemical material after chemical treatment, to measure the petroleum substances

infiltrating the flesh. Unsaturated aliphatic hydrocarbons and aromatic hydrocarbons found in emulsified oil particles were implicated as the compounds responsible for the off-flavor.

Funasaka et al. (1978a) found tetralin, a compound used as a solvent, in Crucian carp (Carassius carassius) and described the carp as having an offensive odor. However, in a subsequent study, Funasaka et al. (1978b) reported that the odor of fish bred in water containing a mixture of volatile fatty acids, aromatic hydrocarbons and phenols was similar to that of fish in natural waters with the offensive odor. They also reported that tetralin caused an odor in bred fish different from the offensive odor in wild fish.

Motohito and Inoue (1973) observed high levels of pristane and $n-C_{17}$ in muscle of Chum salmon (O. keta) contaminated with crude oil. Paradis and Ackman (1975) found that identifiable components (pristane and $n-C_{17}$) were not greatly in excess of the same compounds which were also found to be normal background in organoleptically acceptable lobster meats. They further suggested that the definition of organoleptically acceptable hydrocarbon limits be made on the basis of taste panel assessment of acceptability. Ogata and Miyake (1979) reported that methyl derivatives of benzothiophene, dibenzothiophene and naphthalene from crude oil were transferred to eels. Although they made no mention to the resulting flavor of the eels, previous work by Ogata and Miyake (1973) and Ogata et al. (1977) has revealed the presence of sulfur compounds in petroleum refinery effluent tainted fish.

Pulp and paper mill effluents, sources of off-flavors

Presence of odor in the receiving waters and impairment of fish flavors associated with pulp mill effluents are important problems both to the fishery resources and recreational uses of a waterway thus affected. The identification of and the roles of offending flavor compounds have not been determined to date. Evidence has linked these flavors to pulp and paper mill operations and effluents, but it has not been possible to state which specific compounds or compound classes are responsible.

Ebeling (1931) reported that sulfate mill wastes gave the flesh of fish from the effluent region a resin-like taste which he related to resins and resin acids in the mill waste water. Bergstrom and Vanllin (1937) stated that the condensate from the boiling operations in a sulfate mill caused fish living in the receiving waters to have an unpleasant taste. Jarnefelt (1936) related turpentine to mill wastes to turpentine-like flavors in fish. Motwani and Karamchandani (1956) observed that the waste waters from pulp and paper mills has caused carp to have an offensive smell and to be inedible. Viosca (1952) observed that rate of growth of black moss (Micropterus salmoides) and crappie (Pomoxis sp.) living in a reservoir at a Kraft mill in Springhill, Louisiana, which had recently been discharged and refilled with fresh water from the stream, to be the highest on record. He also rated their flavor to be superior to most wild fish. Choudbury (1958) found sulfates, chlorides and sulfides in the river below a pulp mill and related these substances to unpleasant odors and tastes

in fish taken from the river below the paper mill.

Tamura et al. (1954) studied the effect of two sulphate paper mills on the River Syonai and River Sin, Japan. They concluded that the polluted river water caused the bad tastes and odors in fish in a large area, thus making these fish unfit for consumption. Westfall and Ellis (1940) attributed undesirable flavors in fish (captured below pulp mills on the Rainy River) to absorption of unpleasantly flavored substances from liquid effluents of the pulp mills, and also believed that the decomposition of the disintegrating waste pulp fiber contributed to flavors. They found that the pulp fibers were found two to five miles downstream from the pulp mill. Cole (1935) did not investigate the flavor of black bass (M. solmoides), bluegill (Lepomis pallidis) or yellow perch (Perca flavescens), but he did comment on complaints of poor tasting fish in the area surrounding pulp and paper mills. Cole (1935) did investigate the effects of wood fibers on the fish, and found that as long as fish were active with respiratory movements, wood fibers did not clog the gills or enter the fish body. He also concluded that the death of fish studied near pulp mills was probably due to absorbance of sulfate wastes.

Baldwin et al. (1961) studied the flavor of three species of fish from four different sites in Wisconsin including a cold deep water lake, a shallow mixed-water lake, a flowage site above effluent entry points and a flowage site below effluent entry point. They specifically found that walleye pike (S. vitreum) from below the effluent entry sources to be of poorer flavor quality than those from above

effluent sources. In this instance, pulp and paper mill effluents were implicated in the tainting problem because they were the predominant industry along the flowage. Calbert et al. (1974) observed that the flavor acceptability of walleye pike (S. vitreum) collected above industrial and municipal entrance points along the Flambeau River (Wisconsin) was consistently higher than those obtained from downstream sites. They also noted that the common descriptors applied to off-flavors by panelists were chemical, petroleum-like and metallic rather than characteristic terms indicating the sulfury notes associated with paper mills.

Hasselrot (1964) and German (1968) reported tainting of fish by exposure to pulp and paper mill effluents. Fitterhoff (1963) held rainbow trout (S. gairdneri) in live boxes located downstream from a variety of industrial effluent outfalls to establish the source of off-flavor in Muskegon Lake (Michigan) fish. They found palatabilities of fish held near petroleum refinery and Kraft pulp and paper mill outfalls to be significantly lower than control fish.

Tainting has been observed also in rainbow trout (S. gairdneri) exposed to Kraft pulp mill foul condensate 0.015% (V/V) effluent concentration by Leim and Naish (1979). They also observed that tainting was more pronounced on the left side than the right side when fish were exposed to 0.015-0.138% effluent (V/V). Cook et al. (1973) observed tainting in yellow perch (P. flavescens) captured at immediate paper mill outfall areas, but not two to three miles downstream. They also demonstrated that tainting could be caused by

highly odorous effluent streams from Kraft mill condensates and sulfate waste liquors. Farmer et al. (1973) observed tainting in yellow perch (P. flavescens) exposed to evaporator condensate, digester effluent, recovery furnace flue gas condensate and Kraft mill evaporator condensate, but were unable to demonstrate flavor differences in perch collected from above and below a paper mill discharge on the St. Lawrence River. But when fish were held in cages at the effluent outfall, tainting and death resulted. Whittle and Flood (1977) found that when effluent from a mill employing stream stripping to treat digester blow condensate gases and evaporator condensate was used in tainting studies, a 3% concentration was the lowest level which could be used to produce flavor impairment of yearling rainbow trout within 48 hours. They also reported that tainting would occur during the first 48 hrs of exposure.

Shumway and Chadwick (1971) reported substantial tainting in salmon (O. kisutch) exposed to 1-2% dilutions (V/V) of untreated Kraft mill effluents from a plant with no turpentine recovery facilities. In the same study, salmon exposed to biologically treated effluent concentrations as high as 2.9% (V/V) exhibited little or no flavor impairment. Farmer et al. (1973) reported that 5-10% (V/V) solutions of total Kraft mill effluent concentrations resulted in objectionable flavors in yellow perch. Shumway and Palensky (1973) estimated flavor impairment thresholds of 6% (V/V) for Kraft mill effluent and 30% (V/V) for sulfite waste liquor, but reported that no flavor impairment was observed in rainbow trout exposed to biologically stabilized Kraft

or sulfite mill effluents at concentrations of 8.1% and 33.7% (V/V in clean water), respectively for 72 to 96 hrs.

The variations observed in tainting threshold concentrations can be attributed to different processing conditions in the mills, different effluent stabilization methods used, and the amount of water used in the processing of the pulp. Nonetheless, if proper methods of handling effluents are not used, flavor impairment has been shown to occur within a short period of time. Whittle and Flood (1977) noted that flavor impairment was the most sensitive sublethal response measured in their studies and recommended that it be considered the most critical response to be taken into account when the economic impact of a Kraft mill discharge into a freshwater ecosystem is being evaluated.

The term phenol is used as a collective term for a variety of hydroxyaromatic compounds including phenol, cresols, xylenols, pyrocatechol, resorcinol, hydroquinone, pyrogallol, naphthol, chlorophenols and alkyl substituted phenols. In most references discussing phenols, non-specific methods have been used for the determination of phenol content (Leithe, 1973, as cited by Steinke, 1978).

Many phenol compounds can be found in water and tissue systems, and can be traced to both industrial and natural sources. Hoak (1957a) demonstrated that phenols were present in waters from non-industrial watersheds, and in water with an oak leaf infusion. Hoak (1957b) further demonstrated that natural sources are responsible for

phenolic concentrations of water when he reported that phenol concentrations of waters in the Monongahela and Ohio Rivers increased during periods of heavy rainfall. He concluded that the increased concentration of phenols was due to leaching of phenolics from decaying vegetation.

Individual phenolic compounds differ greatly in their ability to affect the taste and odor of water or fish. Cleary (1960) presented data supplementary to that of Bandt (1955) in which the phenolic concentrations affecting the taste and odor of a variety of fish (trout, carp, eel, perch, roach, minnows, etc.) ranged from 0.1 to 30 ppm for pyrogallol and chlorophenol, respectively. Hoak (1957a) established odor thresholds at 30°C for selected phenolic compounds and these ranged from 10,000 ppb for phenol to 0.33 ppb for phenol to 0.33 ppb for o-chlorophenol. Buttschell *et al.* (1959) reported odor threshold concentrations of 2, 2, and 3 ppb for 2-chlorophenol, 2,4-dichlorophenol, and 2,6-dichlorophenol, respectively. Phenol, 4-chlorophenol, and 2,4,6-trichlorophenol had especially high thresholds (greater than 1,000 ppb).

Damyaneke (1931) demonstrated that fish living in water containing small quantities of phenol developed tarry, phenolic flavors. Westfall and Ellis (1944) confirmed his results. Hoak (1957a) reported that non-specific phenol concentrations increased during periods of heavy rainfall. This increase in phenol concentration was attributed to leaching of phenols from decaying vegetation rather than industrial sources by Hoak (1957b). Böetius (1954) demonstrated that a concentra-

tion of 0.1 ppb chlorophenol in water could be absorbed by eels and give them a carbolic taste. Shumway and Palensky (1973) reported threshold concentrations in rainbow trout (S. gairdneri) for the following phenols: cresol, 70 ppb; m-cresol, 200 ppb; p-cresol, 120 ppb; 2,3-dichlorophenol, 84 ppb; 2,4-dichlorophenol, 1 ppb; 2,4-dichlorophenol, 0.4 ppb in bass and 14 ppb in bluegill; 2,5-dichlorophenol, 23 ppb; 2,6-dichlorophenol, 35 ppb; o-chlorophenol, 60 ppb; p-chlorophenol, 45 ppb; 2,4,6-trichlorophenol, 52 ppb; and guaiacol, 82 ppb. Musty taints in broiler chickens have been shown to be caused by 2,3,4,6-tetrachloroanisole formed by microbial methylation of 2,3,4,6-tetrachlorophenol present in wood shavings (Curtis et al., 1974).

Maga (1978) summarized literature concerning the presence of many simple phenols and phenolic compounds in food including phenol, cresols and other alkyl phenols, guaiacol and other methoxyphenols. For certain foods, including fish, wood smoke composition can have a significant influence on sensory properties. Simple phenols form a major part of the compounds which have been identified (Fiddler, et al., 1966; Wasserman and Fiddler, 1969; and Fiddler et al., 1970). Lignin degradation can be thermally degraded to yield up to 35% mixed simple phenols, however, their composition varies according to the lignin source (Braus and Miller, 1958). Kasahara and Nishibori (1979) reported guaiacol, methyl- and ethylphenol, 2,6-dimethoxyphenol and 4-methyl-2,6-dimethoxyphenol present in the aroma of smoked, cooked salmon. Many alkyl phenols have been identified in the essential oils of spices (Masada, 1978, Sendra and Cūnat, 1980).

Steinke (1978) reported phenol, isopropyl phenols, trimethyl phenols and butyl phenols in tainted Coho salmon (O. kisutch) from Lake Michigan. He also reported phenols in the sediments from Mud Lake (Wisconsin) which has no industrial or municipal effluents entering it. Thus it is apparent that phenols are found in a variety of natural and man-affected situations. Their individual effect on flavor must be evaluated in each individual situation.

Extensive lists of organic compounds found in effluent streams have been published by Fox (1976), Keith (1979), and Peterman et al. (1980), The National Council of the Paper Industry for Air and Stream Improvement (1971; 1979), Hrutfiord et al. (1973) and Hrutfiord et al. (1975). These effluent compounds include saturated and unsaturated fatty acids, and their methyl esters, resin acids and their methyl esters, a large variety of phenolic compounds, monoterpenes, organosulfur compounds, polyaromatic hydrocarbons and phthalates.

Removal of foreign off-flavor causing compounds

Fish exposed to untreated pulp mill effluent will absorb an off-flavor (Shumway and Palensky, 1973; Whittle and Flood, 1977; Cook et al., 1973; Farmer et al., 1973). The removal of these off-flavors can be affected in two ways: purging the flavor compound from the fish, or removal of the potential off-flavor causing compound from the effluent source so these compounds cannot reach the environment.

Several studies have investigated the feasibility of purging off-flavors from fish by holding them in clear running water. Maligalig

et al. (1975) reported complete removal of dimethyl sulfide within 24 hrs and almost complete removal of 2-pentanone within the same time period from channel catfish (I. punctatus). Krishaswami and Kupchanko (1969) observed that rainbow trout (S. gairdneri) lost the oily flavor acquired from exposure to petroleum refinery wastewaters within 96 hrs after transfer to fresh running water. Lobsters held for 5-7 days in clear water environment were only partially purged of low levels of diesel oil contamination resulting from a 12-24 hr exposure (Paradis and Ackman, 1975). Korschgen et al. (1970) reported that tainted carp (Cyprinus carpio) obtained from a location receiving municipal effluent failed to improve in flavor even after holding for 18 days in spring fed and run-off ponds. Removal by purging is at best a lengthy process, and does not appear to be applicable to the sports fishing industry of the Upper Wisconsin River area.

Treatment procedures for effluents are currently available which have been proven capable of eliminating tainting problems if properly used. Biodegradation has been shown to reduce concentrations of mono- and diterpenes, phenols and fatty acids (Rogers, 1978; Leach et al., 1978; Walden and Howard, 1977; and Easty et al., 1978). Volatile sulfides and mercaptans can be removed via oxidation in well aerated lagoons or receiving waters (Leach et al., 1978). Treatment of Kraft mill effluents by aerated lagoons, activated sludge, turbulent contact absorbers (Cook et al., 1973; Farmer et al., 1973) or stream stripping (Cook et al.), reduced objectionable flavors in perch (P. flavescens). Shumway and Chadwick (1971) reported no impairment of flavor in Coho

salmon (O. kisutch) exposed to 2.9% (V/V) solutions of biologically treated Kraft mill effluent. Shumway and Palensky (1973), reported that rainbow trout (S. gairdneri) exposed to biologically stabilized sulfite or Kraft mill effluents at concentrations of 33.7 and 8.1% (V/V), respectively, did not have objectionable flavors.

Additionally, hydrogen peroxide oxidation has been shown to destroy sulfides, mercaptans, thiols, amines, aldehydes and phenols without imparting toxicity or flavors associated with chlorination (Raleigh, 1975). Treatment of effluents with hydrogen peroxide has the additional advantage of adding no contaminants to waterways since any unused peroxide will readily decompose to water and oxygen, and hydrogen peroxide has been used successfully in pulp mills to control odor problems (Raleigh, 1975). Similarly, wet scrubbing with potassium permanganate has been shown to be effective for the removal, via oxidation, of water soluble gases such as hydrogen sulfide, organic sulfur gases and some chlorine compounds, with the formation of odorless, or less offensive compounds (Yang and Cheremisinoff, 1975).

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Chapter 2

IDENTIFICATION OF VOLATILE COMPOUNDS IN FLAVOR-TAINTED
WALLEYE PIKE FROM THE UPPER WISCONSIN RIVER¹

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ABSTRACT

Edible portions of walleye pike (Stizostedion vitreum) from the Upper Wisconsin River system were minced, steam-distilled under reduced-pressure, and ethyl ether extracts of aqueous distillates were analyzed by GC-MS. Evidence for the identity of 112 compounds was obtained, and included were compounds inherent to fish flavors as well as those related to environmental sources. Based on odor assessments of eluting GC fractions, and semi-quantitative comparisons of GC profiles of non-tainted and flavor-tainted fish, alkyl phenols (methyl-, dimethyl-, isopropyl-, diisopropyl-, and methylisopropyl-), aromatic thiols (thiophenol, p-thiocresol, and p-methoxythiophenol), geosmin, and 2-methylisoborneol appear to be significant environmentally-related contributors to flavor-tainting of fish in the section of the river studied.

INTRODUCTION

Flavor taintings of wild fish and shellfish by either natural or industrially-dried chemicals pose esthetic as well as economic threats to commercial and sports fisheries. While earthy and musty flavors caused by streptomycete and algal metabolites have been studied extensively in relation to tainted flavors in fish (1-12), limited definitive chemical information is available on occurrences and causes of other specific flavor taintings in fish. The thermal degradation of dimethyl- β -propiothein to dimethyl sulfide has been reported to cause a petroleum-like off-flavor in canned chum salmon (13) and a blackberry-like off-flavor in Labrador cod (14-16). The flavor compound precursor in these cases was derived from pteropods which were seasonal dietary components.

Kerosene taints found in mullet which had migrated through oil spill polluted waters off the coast of Australia were found to contain volatile hydrocarbon constituents similar to those found in kerosene (17, 18). Mackie et al. (19) found hydrocarbons identical to those present in diesel fuel oil in trout whose flavor was described as having a strong diesel oil taint. Ogata and Miyake (20) concluded that toluene from nearby oil refineries imparted offensive odors to fish and eels captured near Mizushima, Japan. They also found that o-, m-, p-xylenes, benzene, 1-hexene, thiophene, and olefins present in gasoline, and some saturated aliphatic hydrocarbons caused taints in fish. An oily taste in fish from the Bow River in Alberta, Canada, was linked by Krishnaswami and Kupchanko (21) to diluted petroleum waste water.

Ogata et al. (22) reported that eels exposed to a suspension of crude oil in water under laboratory conditions developed an obnoxious odor which they believed to be caused by an accumulation of paraffins (C_{13} - C_{15}), organic sulfur compounds and aromatic hydrocarbons.

Analysis of industrial effluents and natural waters (23-33) for trace organics has shown that large numbers of compounds are potentially available in these environs for contributions to flavor tainting of fish. Intentional exposures of fish to industrial effluents (34-39) and selected compounds found in effluents (39) have been carried out in attempts to determine and characterize flavor-tainting systems. However, definitive associations have been difficult to establish, and in many cases flavor-tainting causes remain obscure (40-43), especially in relation to pulp and paper processing.

This paper presents the results of initial systematic studies on the characterization of volatile compounds present in non-tainted and flavor-tainted walleye pike from a section of the Upper Wisconsin River system influenced by several pulp and paper mills as well as municipalities and other industrial activities. Attention has been focused on compounds with odor qualities indicative of involvement in flavor tainting of fish.

MATERIALS AND METHODS

Sample Collection

Walleye pike (Stizostedion vitreum) were collected from the Wisconsin River by fyke nets or electroshocking by the State of Wisconsin Department of Natural Resources from downstream study sites at Lake Wausau and the Mosinee Flowage and headwaters study site in the Rainbow Flowage (Fig. 1). The first industrialization below the headwaters is located at Rhinelander. Fish ranged from 15 cm and 0.2 kg to 75 cm and 4.5 kg. Fish were iced and air-shipped to the University of Wisconsin-Madison, Department of Food Science. Immediately upon arrival, the fish were filleted and skinned to yield typical edible portions for this species. Fillets were washed with cold running tap water (10°C) vacuum-packaged (less than 125 mm Hg; Multivac Model AG-5 Vacuum Chamber, Sepp Haggemuller KG, Germany) in barrier-type pouches (0.5 mil type 6 Nylon/2 mil Surlyn; American Can Co., Neenah, WI). Fish were then frozen and stored at -25°C until used.

Reduced-Pressure Steam Distillations

Samples of fillets (2 kg) were thawed in sealed packages by submerging in cool water, and then were blended in commercial size Waring Blendor (Model CB-6) with sufficient 10% reagent grade NaCl (MCB Manuf. Chem. Inc., Div. E. Merck, Cincinnati, OH) solution to give slurries with a total volume of 5 L. Slurries were warmed to 100°C for four min to coagulate proteins, and were transferred to a 12 L round-bottom distillation flask. Samples were distilled in a specially constructed, all-glass vacuum distillation apparatus (44)

employing a 12 L sample flask, a 3 L steam generator and a series of 6 dry ice/ethanol and liquid nitrogen cold traps. Samples were slowly degassed, and steam distillation were carried out for four hrs, or until a volume of approximately 3 L of distillate was collected. Steam distillates were thawed, then saturated with reagent grade NaCl, and then were continuously extracted for 16 hrs with 150 ml of ethyl ether (J. T. Baker Chem. Co., Phillipsburg, NJ) in a liquid-liquid extractor designed for solvents less dense than water. In some cases, ethyl ether was first distilled to avoid butylated hydroxytoluene (BHT) present in the ether as an antioxidant.

Ethyl ether extracts were concentrated to approximately 10 ml using a 1.5 cm x 1 m shielded fractional distillation column filled with glass helicies. The fractionation head was electronically controlled for settings of 80% reflux and 20% removal on a 20 second cycle. Concentrated extracts were then transferred to 18 ml Concentratubes (Laboratory Research, Los Angeles, CA). Samples were finally concentrated to a volume of 100-300 µl by removal of the ethyl ether under a gentle stream of nitrogen.

Gas Chromatographic (GC) Conditions

Ethyl ether extracts of the distillates were separated with a Varian Model 1740 gas chromatograph (Varian Associates, Palo Alto, CA) equipped with an effluent splitter (10:90 in favor of the exit) and a flame ionization detector (FID). The effluent splitter allowed simultaneous FID and odor evaluations of eluting components. A 3.6 m x 3 mm i.d. stainless steel column packed with 9% Carbowax 20 M on 100/

120 mesh Chromosorb W AW DMCS was used. Other operational conditions were: nitrogen carrier gas flow rate, 24 ml/min; hydrogen and airflow rates of 24 and 240 ml/min, respectively; injection port temperature, 260°C, and detector temperature, 235°C. Column temperature program rate was 50° to 220°C at 4°C/min.

Extracts from fish from the downstream Mosinee site were also analyzed by a flame photometric detector (FPD) for sulfur compounds and by Hall and electron capture (EC) detectors for chlorinated and other electron-capturing compounds.

A Tracor Model MT 220 gas chromatograph (Tracor Instruments, Houston, TX) equipped with a 1.9 m x 9 mm i.d. glass column packed with 9% Carbowax 20 M was used for FPD analysis of sulfur compounds. Flame conditions were: nitrogen carrier gas flow rate, 43 ml/min; hydrogen, air and oxygen flow rate were 60, 45, and 23 ml/min, respectively; injector temperature was 160°C, and the FPD temperature was 136°C. The column temperature was programmed from 50°C to 220°C increasing at a rate of 8°C/min.

Electron-capture detector gas chromatography (EC-GC) was performed with a Perkin-Elmer Model 34A gas Chromatograph (Perkin-Elmer Corp., Norwalk, CT) equipped with a 1.8 m x 4 mm i.d. glass column packed with a mixed liquid phase of 4% SE-30/6% OV-210 on 80-100 mesh Chrom W-HP. Argon-Methane carrier gas flow rate was 25 ml/min, and other operating conditions were: column temperature, 215°C; injector temperature, 240°C; and detector temperature, 350°C.

Hall detector gas chromatography (45) was performed using a

Varian Model 3700 gas chromatograph (Varian Associates, Palo Alto, CA) equipped with a 1.8 m x 2 mm i.d. glass column packed with 3% OV-101 on 120-140 mesh Gas Chrom Q. Helium carrier gas flow rate was 28 ml/min. The column temperature was programmed from 80° to 240°C at 4°C/min. The operating conditions were: injector temperature, 220°C; furnace temperature, 275°C, and detector base temperature, 240°C.

Gas Chromatography-Mass Spectrometry (GC-MS) Analysis

A Varian Model 1740 gas chromatography (Varian Associates, Palo Alto, CA) coupled with a Du-Pont Model 21-491 B mass spectrometer (Du-Pont de Nemours and Co., Wilmington, DE) with double-focusing and differential pumping capabilities was used. The GC-MS interface system included a single-stage, glass jet-separator. Ion source operating temperature was maintained at 250°C and ionization voltage was 70 eV during scanning. Source pressure ranged from 1×10^{-7} to 1×10^{-5} Torr. A mass scan rate of 4 sec/decade for the mass range from 29 to 800 m/e was used. The instrument was calibrated with a perfluorokerosenes standard. A DS-50 Data Control Nova computer based data acquisition analysis system (Kratos Scientific Instruments, Inc., Westwood, NJ) was used for background subtractions, normalization, and specific mass (m/e) searches.

Compounds were identified by a manual matching of full or partial (8 peak) mass spectra of unknown compounds with published mass spectral data. Primary data sources were the "Eight Peak Index" (46) or the "EPA/NIH Mass Spectral Data Base" (47). Coincidence of unknown and authentic compound retention indices (I_E) (48), and where possible,

authentic compound retention indices (I_E) (48), and where possible, agreements of aromas of eluting unknown compounds with probable authentic compounds were also employed in assigning identities to compounds.

RESULTS AND DISCUSSION

Samples of walleye pike for distillation were selected on the basis of results of sensory analyses of broiled fillets carried out in conjunction with chemical analyses (49). During the course of the investigations several samples from each of the collection sites were distilled, analyzed and studied. These total data have been assembled in summary from Table I with the compounds listed in order of elution from a Carbowax 20 M GC column. Relative peak sizes have been estimated for each compound, but some of these varied between runs as a result of the time of sampling, and as a result of sample handling procedures used during isolation and concentration. However, relative peak sizes were useful in semi-quantitative comparisons of levels of some compounds between samples. Chromatograms shown in Figures 2, 3, and 4 illustrate the general GC patterns obtained from aliquots of flavor extracts from walleyes from the Rainbow Flowage (upstream site), and Lake Wausau and Mosinee Flowage (downstream sites), respectively.

Some peaks in the volatiles from fish obtained from downstream sites had distinct and pronounced odors characterized as phenolic, medicinal, chemical, rubbery, musty and muddy, and these odors were sometimes superimposed with other less distinct or unusual odors. Nevertheless, compounds responsible for these unpleasant odors were considered likely to contribute to the flavor-tainting observed in the downstream fish (49), and were chosen for focused study.

It was apparent that several compounds in the I_R regions of 13.0 - 16.6 were particularly significant because of pronounced,

indole-like, unpleasant, medicinal and phenolic aromas and their abundance in downstream walleye extracts. Mass spectral fragmentation patterns of these compounds indicated the presence of branched, short-chain alkyl phenols, and definitive structural assignments were facilitated by recent reports of Sendra and Cūnat (57, 58) who characterized several isopropyl phenols in the essential oil of Spanish origanum. Development of I_E data for authentic compounds along with odor assessment of known compounds as they eluted from the GC column confirmed the presence of several structurally related alkyl phenols, especially in the downstream walleye pike. However, evidence for very low concentrations of some of the compounds was found in the extracts from upstream fish also. The most abundant and influential alkyl phenols in tainted walleyes appeared to be the 3- and the 4-isopropyl phenols, the 2,4- and the 2,6-diisopropyl phenols, and carvacrol. Characterizing MS fragmentations, Carbowax 20 M I_E values and assessments of aromas of neat samples of alkyl phenols encountered in fish flavor extracts are summarized in Table II.

Carvacrol [2-methyl-5-(1-methylethyl)-phenol] is a relatively common constituent of essential oils (54), and mono-isopropyl phenols have been found recently in the volatiles of tainted Lake Michigan Coho salmon (60). Fox (26) has reported that phenol, guaiacol and 4-methoxy-4-propyl phenol were components of Kraft pulp and paper mill effluent, and Proskin et al. (61) had reported finding phenol, o-, m-, and p-cresol, 2,5-xyleneol, 2,4-xyleneol, and guaiacol in tall oil rectification waste water. Peterman et al. (25) reported the presence of guaiacol, phenol, decyl-, ethyl-, and nonylphenols, phenyl

decane, phenyl dodecane and phenyl undecane, guaiacyl acetone, and chlorinated xylenes and toluenes in sediments from the Lower Fox Watershed. Sheldon and Hites (32, 33) reported phenols, cresols and several partially characterized C₂, C₃, C₄, C₈ alkyl phenols as well as nonyl-, phenyl, cumylphenols in industrially polluted waters of the Delaware River. Thus, it is apparent that some of the malodorous alkyl phenols are likely widely distributed in the environment. Further, it appears probable that they are derived from natural plant constituents, particularly diterpenes (49), and their contributions to fish tainting occurs only when conditions allow development and subsequent accumulation of above threshold concentrations in fish tissue.

Additional compounds with objectionable aromas were observed which eluted from the Carbowax 20 M column generally before alkyl phenols. Mass spectral data strongly suggested aromatic sulfur compounds, but coeluting compounds made definitive assignments difficult. However, analysis of volatiles by selective FPD-GC (Fig. 5) clearly revealed the presence of sulfur compounds in the regions under question. As can be noted in Fig. 5, many sulfur compounds were observed, but those of principal interest were confirmed to be thiophenol, p-thiocresol and methoxythiophenol. General properties of these compounds are included in Table II, and their origin from lignin has been postulated during Kraft or sulfite processing of paper pulp (49). p-Methoxythiophenol has been found in water from the Rhine River (62) and in the volatiles from Coho salmon from Lake Michigan (60), and in both cases the presence of the compound has been attributed to discharges of industrial origin.

While the three sulfur compounds described above, especially thiophenol, appear to contribute greatly to fish tainting in the Wisconsin River, other sulfur compounds appeared to be present in extracts from both non-tainted and tainted samples. These compounds exhibited sulfury, onion-like, broth-like, burnt meat-like and cooked vegetable-like odors. The identification of these compounds was not pursued vigorously because their aromas indicated that they were probably alkyl thiols and disulfides, thiazoles and thiophenes which are commonly found flavor compounds in cooked flesh foods (63, 64). These usually potent flavor compounds arise from the interactions of sulfur-containing amino acids and vitamins with reducing sugars, and a number of them have been reported in volatiles of cooked fish by McGill et al. (65) and Aitken and Connell (66).

Similarly, benzothiazole identified in the study appears to have no significance in fish tainting as it is found ubiquitously in foods. For example, this sulfur-nitrogen heterocyclic has been reported in potatoes, milk and milk products, beef, beer, cocoa and grapes (64). The oxygenated and nitrogen heterocyclic compounds, furans, pyrazines, and pyrroles also result from thermal degradation reactions between reducing sugars and amino acids, and some of these heterocycles can contribute pleasant nutty, roasted, toasted caramel, and burnt type odors to the general aroma profile of food flavor mixtures. Nicotine, a plant alkaloid most commonly associated with tobacco which has been used as an insecticide, was identified in the volatiles of walleye pike from Mosinee Flowage. However, origin of nicotine in this sample is unknown.

The now extensively discussed (1-8, 67) musty and earthy aroma compounds, geosmin and 2-methylisoborneol, were identified in both non-tainted and tainted walleye pike. However, the amount of each in samples varied greatly depending on capture site and season of the year. Fish captured in early summer and from the upstream control site contained the lowest levels, apparently at subrecognition-threshold concentrations. However, detectable levels were observed in fish from all sampling locations during mid to late summer, which corresponds to periods of expected high growth rates for streptomycetes and algae responsible for the production of these substances (67). Thus, it must be acknowledged that geosmin and 2-methylisoborneol contribute directly and substantially to the tainting of fish in the Wisconsin River, and the extent of their involvement in tainting may be influenced by the availability of a direct precursor in the environment (49).

Many other compounds or combinations of compounds that were not identified in this study exhibited unusual aromas as they eluted from the GC column, but the compounds did not have aromas which were not suggestive of the observed tainting. For example, regions exhibiting musty, stale, fish tank-like, pond-like and seaweed-like aromas (I_E 7.6-7.8, 11.4-11.5, 11.6-11.85, 15.7-15.9) were observed. Brunke (68) has reported the identification of substances with similar odors in ambergris, a fragrance ingredient whose origin is in secretions of the sperm whale. Brunke (61) found that structures of compounds with these aroma notes were generally trans-configurations of substituted perhydronaphthofuranes, and this observation could prove useful in later studies.

Several terpenes were identified in the volatiles from walleye pike, and these included the monoterpenes, p-cymene and citronellal, the bicyclic monoterpenes, α -pinene and myrtenal, the macrocyclic monoterpene, α -caryophyllene, and the sesquiterpene alcohol, cadin-4-ene-1-ol (epimer). p-Cymene bears a structural relationship to some of the alkyl phenols identified, and along with p-ocimene has been found in fish volatiles by other (60, 65).

One enantiomer of cadin-4-ene-1-ol has been reported to have an earth, woody aroma, and has been isolated from several streptomyces species (67). The other enantiomer has been isolated from cubeb oil, and has a sweet-spicy aroma (60). Teranishi et al. (69) have summarized some of the evidence for enantiomers have different odors and odor intensities. Generally, it is not unusual to find plant and microbically-derived sesquiterpenes that exhibit different aromas for each enantiomer (68). The GC region in which cadin-4-ene-1-ol as tentatively identified in this study ($I_E = 13.6$ to 14.0) was found to have both sweet, spicy, and heavier soldering rosin-type odors. However, since both enantiomers would be expected to have similar I_E values, it is not possible to comment further on the actual structure(s) of the compound for which analytical data were obtained.

α -Pinene and α -caryophyllene are found in sulfate turpentine fractions (70), and many other terpenes not identified as yet in walleye pike flesh have been reported in pulp mill effluents (26, 71). Thus, the presence of terpenes in fish flavor extracts may be due to both natural accumulation as well as enhanced accumulation caused by

increased concentrations of these compounds in river water from pulping operations. However, the wide distribution of terpenes in wild fish, but their role in fish flavors is undetermined at this time.

Several compounds, especially carbonyls, are readily formed as a result of oxidative deterioration of fish lipids, and the presence of odorous saturated and unsaturated aldehydes ($C_6-C_{11,13}$), acids (C_2, C_6, C_9), ketones and alcohols were not unexpected. Unsaturated fatty acids are also well known compounds in tall oil (72). 2-Methylpent-2-enal ($I_E = 5.31$) was found in all samples, and the branched structure indicates a precursor other than the common fatty acids. Interestingly, it bears a structural relationship to some fragments that would result from the formation of alkyl phenols and earthy compounds from diterpene precursors (49). Similar compounds have also been found in tall oil fractions (71). Benzaldehyde, ethyl benzaldehyde and cyclohexanone have been reported in the steam distilled volatiles of cooked tuna fish (73), and benzaldehyde, phenylacetaldehyde and cyclohexanone were observed in cod by McGill *et al.* (65).

Hept-c,4-enal ($I_E = 6.33$) has been reported by McGill *et al.* (65). to contribute to the fish meal, cardboard odor, and taste of cold stored cod. It has also been implicated as responsible for fishy flavors in soybean oil (74). Hept-t,2-enal and hept-t,2,c,4-dienal have also been associated with fishy, cardboardy flavors caused by lipid oxidation (65). Swoboda and Peers (75) reported that the fishy aroma impact of the 2,4-dienals is probably due to a complex sensation of the aroma of decadienal and decatrienal compounds rather

than an individual compound. Old fish oil and old fried greasy potato aromas eluted in the region $I_E = 9.9$ to 10.0 , and nonadienal was positively identified in this same region. However, this association was obscured by other compounds because neat nonadienal has a melon-like, cucumber flavor (76). A mushroom-type odor was observed in the GC effluent at $I_E = 6.7$, and was associated with oct-1-ene-3-one. This compound has been shown to be an oxidation product of linolenic and more highly unsaturated fatty acids (77).

Damascenone ($I_E = 11.68$) and the related compounds α -ionone ($I_E = 12.25$), β -ionene ($I_E = 13.10$) and pseudo-ionone ($I_E = 12.00$) were found in all fish, and these compounds have pleasant, floral odors. They have been found in several foods and beverages (78, 79), and all are believed to be oxidation products of carotene or carotene-like precursors (78, 79). The role of these compounds in the flavors of walleye pike is undetermined at this time. Two compounds, indene and indanone (fused C_6 -aromatic- C_5 -cycloalkene or corresponding cycloalkane keto derivative, respectively) which have structural similarities to the ionones were also identified. Indene, $I_E = 8.61$, was associated with a sharp chemical odor, and has been reported in cranberries, beef, coffee, filberts and peanuts (64) as well as in coal tar (80). Indanone, $I_E = 14.41$, was associated with a GC peak having an indole-like, alkyl phenol-like aroma. These fused-ring compounds could result from ring closures of precursor compounds with structures like the ionones.

Pronounced woody, pine-lumber-like, and soldering rosin smoke-like odors were observed during elution of volatiles from all samples

(I_E regions 12.5 to 12.8, 14.0 to 14.1, 14.3 to 14.9 and 15.5 to 15.9), but the downstream sites usually yielded fish with more abundant amounts of these compounds which generally had pleasant aromas. Authentic reference compounds with these aroma characteristics were not available, but pyrolyzed rosin which is composed chiefly of diterpene resin acids yielded a smoke condensate with a complex mixture of compounds that included many with wood-like, propolis-like and rosin smoke-like odors that were similar to those observed in the fish extracts. Compounds in the rosin smoke condensate with parent ions of m/e 168, 192 or 196 appeared to be responsible for strong woody rosin-type odors. Based on structural deductions from strong mass spectral fragmentation patterns, one compound with a distinct rosin smoke-like odor eluting at $I_E = 15.15$ to 15.9 from a Carbowax 20 M column was tentatively identified as 2-aceto-5-isopropyl-cyclopent-1-enol [m/e 71 (100%), 43 (95%), 97 (90%), 57 (85%), 111 (80%), 125 (60%), M^+ 168 (40%), 153 (30%), 151 (20%)]. This compound could be formed from a thermally-induced fragmentation and rearrangement of abietic acid. However, only a very tentative mass spectral pattern for this compound could be extracted from MS data for the walleye pike extracts, and the influence of this group of compounds on the flavor of fish remains undetermined at this time.

The number of alkanes (C_{10} , C_{15} , C_{17} , C_{20} , and C_{23}) were identified in fish samples from all three study sites. These saturated high molecular weight hydrocarbons generally exhibit little odor, and it is doubtful that they contribute to the flavor of fish. Similar hydrocarbons have been reported in fish by McGill et al. (65)

and Ackman (81), Steinke and Lindsay (60) and Welch et al. (82). Even though hydrocarbons were suspected, Paradis and Ackman (83) reported that hydrocarbon concentrations were similar in both diesel fuel-tainted and organoleptically acceptable lobster meats. It is likely that the alkanes observed in the current study arose from the biological food chain in keeping with Welch et al. (82) who have proposed that substantial background concentrations of alkanes are present naturally in fish.

Naphthalene, methyl naphthalene, biphenyl and 3-methyl biphenyl were reported in sediments and water from the Lower Fox River Watershed by Peterman et al. (25). In the current study biphenyl and 3-methyl biphenyl were identified and each gave sweet, pleasant odor impressions as they eluted from the gas chromatographic column. Naphthalenes have been found in numerous foods as well as in samples from industrial sources (23, 31, 64). It is doubtful, however, that sufficient quantities of these compounds were present to contribute to tainting-flavors observed in fish from the study sections of the river.

Some chlorinated phenolic compounds have pronounced medicinal aromas (39), and have been frequently identified in waters containing effluents from industrial and municipal sewage treatment plants (23, 25, 26). Such compounds have been linked to odor problems in water (84-87), and have been found in numerous tissues (25). However, in the current investigation, GC-MS analysis of flavor extracts using a Carbowax 20 M column for separation did not yield spectra strong enough to be interpreted as those for chlorinated

compounds. EC and Hall detectors that are used in the selective GC analysis of halogenated pollutants (88) were employed in attempts to detect the low levels chlorinated compounds present in fish extracts. GC patterns for both the EC and HD analyses of volatile extracts of walleye pike from the Mosinee Flowage are shown in Fig. 6 and 7, respectively.

The retention times of the earlier peaks in the mixed SE-30/OV-210 EC-GC run (Fig. 6) were indicative of polychlorinated biphenyls (PCB's), probably arochlor 1248 and 1254 which have been identified in the Wisconsin River (89). The later peaks in this EC-GC run correspond to the region which phthalate esters elute from this column, and diethyl-, diisobutyl-, and dioctyl phthalate were identified earlier by GC-MS of extracts of walleye pike separated on the Carbowax 20 M column. Phthalate esters appear to be nearly ubiquitous in the environments, and like PCB's have been reported to accumulate in fish (90, 91). The absence of peaks in the early region of the OV-101 Hall detector-GC run (Fig. 7) was significant from a flavor-tainting stand point, however, as this is the region where mono- and through pentachlorophenols elute from this column. Thus, it can be concluded that chlorophenols were not a factor in the flavor-tainting of fish in the Upper Wisconsin River that were used in this investigation.

In summary, this paper has focused principally on the identification and general discussion of sources of volatiles found in walleye pike from the Upper Wisconsin River. Potentially significant tainting compounds have been identified as aromatic thiols and alkyl phenols. The flavor properties of these compounds along with proposed precursors are discussed in detail in a following paper (49).

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Table I. Summary of Volatile Compounds Identified in Walleye Pike from the Upper Wisconsin River.

RETENTION INDEX ¹ (I _E)		SOURCE OF WALLEYED PIKE SAMPLES									
		RAINBOW FLOWAGE (HEADWATERS)			MOSINEE FLOWAGE (DOWNSTREAM)			LAKE WAUSAU (DOWNSTREAM)			
		Carbowax 20M EXP.	PROBABLE COMPOUND (I _E /M.S.)	PRES- ENCE ²	REGION ODOR FROM CHROMATOGRAPH ³	REL- ATIVE PEAK SIZE ⁴	PRES- ENCE ²	REGION ODOR FROM CHROMATOGRAPH ³	REL- ATIVE PEAK SIZE ⁴	PRES- ENCE ²	REGION ODOR FROM CHROMATOGRAPH ³
2.0 to 2.3	(2.33) ^a	Ethyl acetate	+	sweet, estery	VL	+	fruity, estery	VL	+	estery	VL
2.6	(2.65)	Methyl propionate	+	brothy	L	+	brothy	L	+	brothy	L
4.0 to 4.3	(4.00) ^b	α-Pinene and a Methyl silane	-			+	chemical, sharp	M	+	solvent, sharp	L
4.4 to 4.7	(4.53)	Hexanal	+	green, grassy	M	+	grassy	L	+	planty, green	M
4.7 to 5.0		Unknown		skunky	M		skunky	M		skunky	M
5.3	(5.31)	2-Methyl-pent-2-enal	+	planty	L	+	spicy, green	L	+	spicy	L
5.6	(5.63)	Heptanal	+	planty	L	-	green, sharp	S	+	sharp	S
5.8	(5.76)	Dodecane	+	chemical	L	+	chemical	S	-		
5.8		Unknown		chemical	L		chemical	S		stale, plastic	S
5.9	(5.90)	β-Ocimene	+	planty, chemical	VS	+	planty	M	+	planty	S
6.0 to 6.1	(6.15) ^c	Methyl thiazole	+	green, planty	VS	+	planty	VS	+	planty	VS
6.0 to 6.1	(6.09)	cis-4-Heptenal	+	sharp	VS	+	sharp	M	+	sharp	S
6.4	(6.45)	p-Cymene	-			+	unpleasant, sharp	S	-		
6.4 to 6.7	(6.41) ^d	2-Methyl pyrazine	+	popcorn, meaty	VS	-			+	nutty, pyrazine-like	VS

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			PRES- ENCE ²	REGION ODOR FROM CHROMATOGRAPH ³	REL- ATIVE PEAK SIZE ⁴	PRES- ENCE ²	REGION ODOR FROM CHROMATOGRAPH ³	REL- ATIVE PEAK SIZE ⁴	PRES- ENCE ²	REGION ODOR FROM CHROMATOGRAPH ³	REL- ATIVE PEAK SIZE ⁴
EXP.	Carbowax 20M (REF.)	PROBABLE COMPOUND (I _E /M.S.)									
6.5 to 6.6	(6.58)	Octanal	+	waxy, soapy	M	+	waxy, soapy	L	+	waxy, soapy	M
6.6 to 6.8	(6.70)	Oct-1-en-3-one	±	mushrooms	S	±	mushrooms, musty	S	±	musty, mushrooms	VS
6.6 to 6.8	(6.84)	Hept-t ₂ -enal	±	aldehyde, sharp	M	±	aldehyde, green	L	±	unsaturated aldehyde	M
7.29	(7.06)	Ethyl pyrazine	-			±	unpleasant, burnt	VS	-		
7.2 to 7.3	(7.23) ^e	4-Ethyl-2-methyl thiazole	±	green, planty, unpleasant	VS	±	myrcene-like	VS	-		
7.4	(7.56) ^e	Nonanal	±	aldehyde, soapy	VS	-			-		
7.5 to 7.6	(7.55) ^c (7.55) ^d	Dimethyl thiazole 2-Ethyl-6-methyl pyrazine	-			±	nutty, sharp, peanutty	VS	-		
7.6	(7.70)	2,4-Hexandienal	±	planty	VS	-			-		
7.6 to 7.8	(7.79) ^c	2-Butylthiazole	±	green, oniony	VS	-			-		
7.6 to 7.8	(7.74) ^c	Ethoxythiazole	±	unpleasant	VS	±	unpleasant, stale fish tank	VS	±	stale fish tank	VS
7.9		Unknown	-				pyrazine, green	VS			
7.8 to 7.9	(7.90)	Acetic Acid	+	unpleasant	VS	+	unpleasant, stale fish tank	VS	+	stale fish tank	VS
8.0 to 8.1	(7.81) ^c	Isobutylthiazole	±	green tomatoes, green	M						

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		EXP.	Carbowax 20M (REF.)	PROBABLE COMPOUND (I _E /M.S.)	PRES- ENCE ²	REGION ODOR FROM CHROMATOGRAPH ³	REL- ATIVE PEAK SIZE ⁴	PRES- ENCE ²	REGION ODOR FROM CHROMATOGRAPH ³	REL- ATIVE PEAK SIZE ⁴	PRES- ENCE ²	REGION ODOR FROM CHROMATOGRAPH ³
8.0 to 8.1	(8.24) ^d	2,3-Diethylpyrazine	+	musty	VS	+	pyrazine	S	-			
8.04 to 8.2	(8.15)	Oct-t,2-enal	+	aldehyde	S	-			-			
8.4 to 8.5	(8.07)	4-Vinyl pyridine	+	burnt	S	+	pyridine-like	S	+	green		VS
8.4	(-)	Trimethyl pentene	-			-			+	green, celery		VS
8.43 to 9.09	(8.33)	Methional	+	aldehyde-like	S	+	boiled potatoes	S	+	green, musty		S
8.49	(8.49) ^e	Citronellal	-			+	unpleasant, plant, musty	S	+	slight celery musty, green		S
8.6	(8.61)	Indene	-			+	sharp, chemical	M	-			
8.7 to 8.8	(8.71) ^b	Benzaldehyde	+	chemical, burnt	L	+	sharp, chemical unpleasant	L	+	burnt		L
8.79	(8.72)	Thiophenol	-			+	heavy, sharp chemical, unpleasant	L	+	sharp, chemical		S
8.80	(8.77)	Pentadecane	+	burnt	L	+	heavy, not pleasant	L	-			
8.8 to 8.9	(8.89)	2-Isobutyl-3-methoxyl- pyrazine	+	bell pepper	S	+	bell pepper	S	+	green, peanut-like		S
8.9 to 9.0	(8.97)	Furfural acetate				+	solvent-like burnt		+	burnt		

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9.0 to 9.1	Unidentified (m/e 140) (odor unknown)		green, planty, cooked vegetable	L		green, planty, cooked vegetable	L		green, planty, cooked vegetable	L
9.0	(8.85) ^c 2,5 Diethyl-4-methyl thiazole	+	green tomato leaves							
9.01 to 9.3	(9.51) ^c p-methoxythiazole (9.10) ^d Diethyldimethyl pyrazine	+	cooked vegetables	S	+	roasted	S	+	cooked vegetables	S
9.2 to 9.3	(9.10) Non-t,2-enal	+	pleasant, aldehyde-like	S	+	cucumbers	S	+	floral, green vegetables	S
9.3	(9.0) 5-Methyl-2-furfural	+	cooked vegetables	M	+	cooked celery	S	+	cooked green vegetables	S
9.3 to 9.4	Unidentified (m/e 192)		cooked vegetables, bell pepper	M		roasted, bell pepper cooked, celery	S		cooked carrots green vegetables	S
9.6 to 9.7	(9.68) Undecanal	+	camphoraceous, chemical		+	musty, unpleasant	S	+	unpleasant chemical	S
9.6 to 9.7	(9.66) 2-Methylisobornol	+	camphoraceous, chemical	L	+	musty, unpleasant	S	+	musty	S
9.6 to 9.8	Unidentified cyclohexenone (m/e 110)	-			+	objectionable, sulfury	S	+	objectionable, green	S
9.6 to 9.8	(9.75) p-Thiocresol	+	heavy chemical	S	+	objectionable, sulfury		+	objectionable, green	S
9.9 to 10.0	(9.70) Nona-t,2-c,6-dienal	+	odor not noted	M	+	odor not noted	S	+	odor not noted	S
9.9 to 10.0	Unknown		burnt chemical	M		old fish oil, old fried greasy potatoes	S		unpleasant	S

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		EXP.	PROBABLE COMPOUND (I _E /M.S.)	PRES- ENCE ²	REGION ODOR FROM CHROMATOGRAPH ³	REL- ATIVE PEAK SIZE ⁴	PRES- ENCE ²	REGION ODOR FROM CHROMATOGRAPH ³	REL- ATIVE PEAK SIZE ⁴	PRES- ENCE ²	REGION ODOR FROM CHROMATOGRAPH ³
10.1 to 10.3	(10.14)	Phenylacetaldehyde	+	very unpleasant, chemical, stale	M	+	green, like phenylacetaldehyde	S	+	unpleasant, cheesy	S
10.1 to 10.3		Unidentified (m/e 172)					old grease, green, like phenylacetaldehyde	S			
10.4		Unknown		vegetable pleasant			planty	S		slight floral	S
10.4 to 10.5	(10.42)	Myrtenal	+	harsh	M	+	cooked celery, green, unpleasant	S	+	slight floral unpleasant	S
10.4 to 10.6	(10.33)	2,6,6,9-Tetramethyl- (E,E,E)-1,4,8- cycloundecatriene (α -Caryophyllene or Humulene)	+	harsh	M	+	unpleasant, chemical	S	+	strange chemical	S
10.5		Unidentified (m/e 164)						S		slight floral, unpleasant, strange chemical	S
10.6 to 10.9		Unknown	+	planty, oniony	M	+	toasted, roasted	L	-		
10.7	(10.66)	m-Ethyl benzaldehyde	+	licorice, anise like	M	-			-		
10.7 to 10.8	(10.75) ^e	Heptadecane	-			+	burnt, musty, chemical, unpleasant	L	+	strange chemical unusual, heavy	L
10.7 to 10.9		Unknown		burnt, harsh chemical	M		chemical, burnt, musty unpleasant	L		strange chemical heavy, burnt	L
10.8 to 10.9		Unknown					floral	L		slight floral	L

Table I. Summary of Volatile Compounds Identified in Walleye Pike from the Upper Wisconsin River.

RETENTION INDEX ¹ (I _E)		SOURCE OF WALLEYED PIKE SAMPLES										
		RAINBOW FLOWAGE (HEADWATERS)			MOSINEE FLOWAGE (DOWNSTREAM)			LAKE WAUSAU (DOWNSTREAM)				
		EXP.	Carbowax 20M (REF.)	PROBABLE COMPOUND (I _E /M.S.)	PRES- ENCE ²	REGION ODOR FROM CHROMATOGRAPH ³	REL- ATIVE PEAK SIZE ⁴	PRES- ENCE ²	REGION ODOR FROM CHROMATOGRAPH ³	REL- ATIVE PEAK SIZE ⁴	PRES- ENCE ²	REGION ODOR FROM CHROMATOGRAPH ³
10.9	(10.95)	p-Ethyl benzaldehyde	-			+	planty, green	L	-			
10.9 to 11.0	(10.90)	p-Cresyl acetate	+	licorice, anise	S	+	licorice	L	+	burnt		S
11.1	(11.14)	1-Octyl benzene	-			+	sheepy, phenol	M	-			
11.1 to 11.2	(11.12)	Naphthalene	-			+	burnt, heterocyclic, heavy chemical	M	+	burnt		S
11.1 to 11.3		Unknown		burnt vegetable	L		burnt, heterocyclic, heavy chemical	M		burnt		S
11.4		Unknown					slight guaiacol smokey	S				
11.4 to 11.5	(11.42) ^d	2-Ethyl-6,7-dihydro-5H- cyclopentapyrazine	+	nutty, pyrazine	L	+	toasted, nutty	M	+	unpleasant, musty, green		M
11.4		Unknown		stewed vegetables	L		stewed vegetables	S				
11.6		Unknown		meaty, vegetables	M		green	M	-			
11.66		Unknown		thiazole-like burnt, harsh	M							
11.6 to 11.75	(11.70) ^f	Deca- <u>t</u> ,2- <u>e</u> ,4,7-trienal	-			+	unpleasant, musty	S	+	unpleasant musty		S
11.6 to 11.85	(11.81)	Hexanoic Acid	+	chemical, burnt	S	+	musty, burnt heavy, unpleasant	S	+	unpleasant, sharp		S
11.78		Unknown								cooling, minty		S

Table I. Summary of Volatile Compounds Identified in Walleye Pike from the Upper Wisconsin River.

RETENTION INDEX ¹ (I _E)			SOURCE OF WALLEYED PIKE SAMPLES								
			RAINBOW FLOWAGE (HEADWATERS)			MOSINEE FLOWAGE (DOWNSTREAM)			LAKE WAUSAU (DOWNSTREAM)		
			Carbowax 20M EXP.	PROBABLE COMPOUND (I _E /M.S.)	PRES- ENCE ²	REGION ODOR FROM CHROMATOGRAPH ³	REL- ATIVE PEAK SIZE ⁴	PRES- ENCE ²	REGION ODOR FROM CHROMATOGRAPH ³	REL- ATIVE PEAK SIZE ⁴	PRES- ENCE ²
11.7 to 11.8	(11.78) Tridecanal	-			+	aldehyde, soapy	S	+	sharp, green	S	
11.8 to 11.9	(11.68) 2,6,6-Trimethyl-1-crotonyl- 1,3-cyclohexadiene (Damascenone)	+	roses	S	+	roses	S	+	roses	S	
12.0	(12.00) 6,10-Dimethyl-3,5,9- undecatriene-2-one (pseudo-Ionone)	+	floral	S	+	floral	S	+	floral	S	
12.02	(12.04) Geosmin	+	earthy, beet-like	S	+	earthy, beet-like	S	+	earthy, muddy beet-like	S	
12.1 to 12.2	(12.09) Methyl naphthalene	+	earthy, beet-like	S	+	earthy, slight floral, beet-like	S	+	earthy, muddy, beet-like, sweet	S	
12.1	Unidentified (m/e 220)					earthy, slight floral, beet-like	S				
12.1	Unidentified (m/e 240)					earthy, slight floral, beet-like	S				
12.2	Unidentified (m/e 124)				-			+	earthy, muddy beet-like, sweet	S	
12.2 to 12.3	(12.21) o-Methoxyphenol	+	burnt	S	+	burnt	S	+	earthy, muddy beet-like, sweet	S	
12.2 to 12.3	(12.25) 4-(2,6,6-trimethyl-2-cyclohex-1-yl) 3-Buten-2-one (α-Ionone)	-			+	semi-floral	S	+	sweet, muddy	S	
12.3 to 12.4	(12.40) 3-(1-methyl-2-pyrrolidinyl)- pyridine (Nicotine)	-			+	burnt plant	S	-			

Table I. Summary of Volatile Compounds Identified in Walleye Pike from the Upper Wisconsin River.

RETENTION INDEX ¹ (I _E)		SOURCE OF WALLEYED PIKE SAMPLES									
		RAINBOW FLOWAGE (HEADWATERS)			MOSINEE FLOWAGE (DOWNSTREAM)			LAKE WAUSAU (DOWNSTREAM)			
		Carbowax 20M EXP. (REF.)	PROBABLE COMPOUND (I _E /M.S.)	PRES- ENCE ²	REGION ODOR FROM CHROMATOGRAPH ³	REL- ATIVE PEAK SIZE ⁴	PRES- ENCE ²	REGION ODOR FROM CHROMATOGRAPH ³	REL- ATIVE PEAK SIZE ⁴	PRES- ENCE ²	REGION ODOR FROM CHROMATOGRAPH ³
12.3 to 12.4	(12.42)	p-Methoxythiophenol	-	burnt	S	+	burnt plant	S	+	sweet, muddy	S
12.3 to 12.4	(12.34) ^e	Octadecane	+	burnt	S	+	burnt plant	S	+	sweet, muddy	S
12.4 to 12.5	(12.40) ^g	Phenethyl propionate	-			+	burnt plant	S			
12.4 to 12.6		Unknown		wet, papery	S						
12.5 to 12.7		Unidentified		woody, kitchen matches	L		woody, sawed pine planty	VL		pine lumber	VL
12.68		Unknown		heavy chemical	L						
12.6 to 12.8	(12.74)	2,6 di-tert-Butyl-4-methyl phenol (BHT; artifact from ether)	+	woody, kitchen matches	VL	+	sawed pine woody, planty	VL	+	pine lumber	VL
12.9		Unknown		planty	M						
13.0		Unknown		liver, meaty	S						
13.0 to 13.1	(13.08)	o-Cresol		stale, heavy	M	+	chemical	VL	+	unpleasant	M
13.1	(13.10)	2,6 Diisopropyl phenol				±			±		
13.1	(13.10)	1,2,3,4-tetrahydro-1,6- trimethyl naphthalene (β-Ionene)	±	sweet	M	±	floral, jam	M	±	heavy, sweet	M

Table I. Summary of Volatile Compounds Identified in Walleye Pike from the Upper Wisconsin River.

RETENTION INDEX ¹ (I _E) Carbowax 20M EXP. (REF.)			SOURCE OF WALLEYED PIKE SAMPLES								
			RAINBOW FLOWAGE (HEADWATERS)			MOSINEE FLOWAGE (DOWNSTREAM)			LAKE WAUSAU (DOWNSTREAM)		
			PROBABLE COMPOUND (I _E /M.S.)	REGION ODOR FROM CHROMATOGRAPH ³	REL- ATIVE PEAK SIZE ⁴	PRES- ENCE ²	REGION ODOR FROM CHROMATOGRAPH ³	REL- ATIVE PEAK SIZE ⁴	PRES- ENCE ²	REGION ODOR FROM CHROMATOGRAPH ³	REL- ATIVE PEAK SIZE ⁴
13.2 to 13.4	(13.31) Benzothiazole	rubbery, old tennis shoes	S	+	hot rubber old tennis shoes	S	+	rubbery		S	
13.3	(13.38) Phenol			-	hot rubber old tennis shoes	S	+	rubbery		S	
13.3	Unidentified (m/e 140) (odor unknown)			-	hot rubber old tennis shoes	S		rubbery		L	
13.4	Unknown	burnt, slight floral	S	-							
13.4	Unknown			-	stale, bad pond	VL					
13.5	(13.48) Dimethyl naphthalene	(no odor)	S	+	(no odor)	S	+	(no odor)		S	
13.5	(13.50) Biphenyl	sweet, ripe berries	VL	+	tire patch stale, bad pond	L	+	burnt rubber		L	
13.5 to 13.6	Unidentified (m/e 220) (odor unknown)				tire patching alkyl phenol-like	L		burnt rubber		L	
13.6 to 13.8	(14.00) Cadin-4-ene-1-ol (13.60) gamma Nonalactone	sweet, ripe berries	VL	±	lactone-like coconutty	M		sweet vanillin-like veratrole-like		M	
13.8 to 14.0	(13.69) ^b t-Cinnamaldehyde Unidentified (m/e 180)	sweet, spicy, floral	L	±			±	cloves		L	
13.73	(13.73) Eicosane	(no odor)	VL	+	(no odor)	L	+	(no odor)		L	
13.8	(13.90) Tridecanol	(no odor)	VL	+	(no odor)	M	+	(no odor)			
13.8	(13.91) Octanoic Acid			-	odor not noted	M					

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RETENTION INDEX ¹ (I _E)		SOURCE OF WALLEYED PIKE SAMPLES								
		RAINBOW FLOWAGE (HEADWATERS)			MOSINEE FLOWAGE (DOWNSTREAM)			LAKE WAUSAU (DOWNSTREAM)		
		PRES- ENCE ²	REGION ODOR FROM CHROMATOGRAPH ³	REL- ATIVE PEAK SIZE ⁴	PRES- ENCE ²	REGION ODOR FROM CHROMATOGRAPH ³	REL- ATIVE PEAK SIZE ⁴	PRES- ENCE ²	REGION ODOR FROM CHROMATOGRAPH ³	REL- ATIVE PEAK SIZE ⁴
Carbowax 20M EXP. (REF.)	PROBABLE COMPOUND (I _E /M.S.)									
13.9 to 14.0	(13.98) ^f m-Cresol	-			+	cresol-like	S	+	alkyl phenol	S
14.0 to 14.1	Unidentified (m/e 178)		indole-like, medicinal	S		indole-like, alkyl phenol	L		alkyl phenol	L
14.0 to 14.1	Unidentified (m/e 192)		indole-like, medicinal	S		indole-like alkyl phenol	L		alkyl phenol	L
14.0 to 14.1	(14.15) 2-Isopropyl phenol				+	bad indole alkyl phenol	L	+	alkyl phenol	L
14.0 to 14.1	(14.16) ^e p-Cresol				+	bad indole alkyl phenol	L	±	alkyl phenol	L
14.0 to 14.1	(14.14) Indanone	±	indole-like medicinal	S	±	bad indole alkyl phenol	L	±	alkyl phenol	L
14.0 to 14.1	Unknown		weak, rosiny	L	-				soldering rosin	L
14.1	(-) 1,5-di-tert-Butyl-dimethyl- bicyclo (3.1.0) hexanone	-			±	bad indole alkyl phenol	L	±	alkyl phenol	L
14.1	(14.00) 2,4-Dimethyl phenol	-			+	bad indole alkyl phenol	L			
14.1 to 14.3	Unknown		floral, sweet	M						
14.3 to 14.5	Unidentified (m/e 196)		floral, sweet	M		soldering rosin	L		soldering rosin	L
14.5	Unknown		floral, vanilla-like	S					chemical	L

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RETENTION INDEX ¹ (I _E)		SOURCE OF WALLEYED PIKE SAMPLES								
		RAINBOW FLOWAGE (HEADWATERS)			MOSINEE FLOWAGE (DOWNSTREAM)			LAKE WAUSAU (DOWNSTREAM)		
		Carbowax 20M EXP. (REF.)	PROBABLE COMPOUND (I _E /M.S.)	PRES- ENCE ²	REGION ODOR FROM CHROMATOGRAPH ³	REL- ATIVE PEAK SIZE ⁴	PRES- ENCE ²	REGION ODOR FROM CHROMATOGRAPH ³	REL- ATIVE PEAK SIZE ⁴	PRES- ENCE ²
14.5 to 14.9	Unidentified (m/e 196)	+	hot, rosiny	VS	+	rosiny, lumber	VL	+	rosin-like, chemical in tail	VL
14.6 to 14.7	(14.64) 3-Methyl biphenyl	+	hot, rosiny	VS	+	rosiny, lumber	VL	+	chemical in tail	
14.6	Unknown		celery-like	S	-			-		
14.7	Unknown		chemical, burnt	M		stale, musty, rubbery air	VL		bad	M
14.7 to 14.8	(14.73, 2,5- and 2,3- 14.84) Dimethyl phenols	+	chemical, burnt	M	+	stale, musty rubbery air	VL	+	bad	M
14.8 to 14.9	(14.87) ^e Heneicosane	+			+			+		
14.7 to 14.9	(14.87) ^e 5-Methyl-2-(1-methyl ethyl)- phenol (Thymol)	+	chemical, burnt	M	+	stale, musty, rubbery air	VL	+	bad	M
14.9 to 15.0	Nonanoic acid	+	odor not noted		+	odor not noted		+	odor not noted	
14.9 to 15.1	(14.91) Unknown		pleasant, cooked	S		burnt sugar, sweet	M		sweet burnt jam	M
15.00	(15.00) Ethyl pentadecanoate	+	pleasant, cooked	S	+	burnt sugar, sweet	M	+	sweet burnt jam	M
15.2 to 15.3	(15.27, 15.34) 3- and 4-Isopropyl phenols	+	mild indole	S	+	phenolic	L	+	medicinal	L
15.2 to 15.3	(15.34) 3,4-Dimethyl phenol	+	mild indole	S	+	phenolic	L	+	medicinal	L

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RETENTION INDEX ¹ (I _E)		SOURCE OF WALLEYED PIKE SAMPLES									
		RAINBOW FLOWAGE (HEADWATERS)			MOSINEE FLOWAGE (DOWNSTREAM)			LAKE WAUSAU (DOWNSTREAM)			
		Carbowax 20M EXP. (REF.)	PROBABLE COMPOUND (I _E /M.S.)	PRES- ENCE ²	REGION ODOR FROM CHROMATOGRAPH ³	REL- ATIVE PEAK SIZE ⁴	PRES- ENCE ²	REGION ODOR FROM CHROMATOGRAPH ³	REL- ATIVE PEAK SIZE ⁴	PRES- ENCE ²	REGION ODOR FROM CHROMATOGRAPH ³
15.2 to 15.3	(15.41) ^e	2-Methyl-5-(1-methyl ethyl)-phenol (carvacrol)	-			+	phenolic	L	+	medicinal	L
15.5		Unknown		stale, musty, dusty	M	-			-		
15.5 to 15.6	(15.49)	Docosane	+	no odor	M	+	no odor		+	no odor	
15.5 to 15.9	(-)	2-aceto-5-isopropyl- cyclopent-1-enol		spicy	S		woody, piney, rosiny	VVL		piney, rosiny	VVL
15.5 to 15.9		Unidentified terpene derivative (m/e 194) (odor unknown)		spicy	S		woody, piney, rosiny	VVL		piney, rosiny	VVL
15.5 to 15.9		Unidentified (m/e 196)					woody, piney rosiny	VL		piney, rosiny	VVL
15.6	(15.65)	Methyl hexadecanoate	+	little odor	S	+	little odor	VS	+	little odor	L
15.6	(15.55)	2,4-Diisopropyl phenol	+	heavy chemical	S	+	heterocyclic-like	VS	+	piney, naphthalic	L
15.7 to 15.9		Unidentified (m/e 196) (odor unknown)		cooked vegetables, musty	S		woody aldehyde	S		musty, camphor	S
16.5 to 16.6	(16.58)	3,5-Diisopropyl phenol	+	chemical	VS	+	cleaning compound, iodine-like	M	+	iodine-like, musty	M
16.6	(16.66)	Diethyl phthalate	+	phthalate-like	L	+		S	+		M
16.6 to 16.7	(16.63)	Methyl heptadecanoate	+	little odor	L	+	little odor	S	+	little odor	M
16.7 to 16.8		Unknown		plant-like	S						

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		RAINBOW FLOWAGE (HEADWATERS)			MOSINEE FLOWAGE (DOWNSTREAM)			LAKE WAUSAU (DOWNSTREAM)		
		EXP.	PROBABLE COMPOUND (I _E /M.S.)	PRES- ENCE ²	REGION ODOR FROM CHROMATOGRAPH ³	REL- ATIVE PEAK SIZE ⁴	PRES- ENCE ²	REGION ODOR FROM CHROMATOGRAPH ³	REL- ATIVE PEAK SIZE ⁴	PRES- ENCE ²
16.7 to 16.8	Unknown		burnt hair	VS	-					
16.8 to 16.9	Unknown					heavy chemical	M			
17.0	(17.00) Ethyl heptadecanoate	+	little odor	S	+	little odor	L	±	little odor	S
17.1	Unknown					planty, hot, sharp	M			
17.1 to 17.41	Unknown		sweet	S		sweet, pleasant	M		burnt vanillin	M
17.5	(17.58) Methyl octadecanoate	+	little odor	M	+	little odor	M	+	little odor	M
17.6	Unknown					hot	L		sweet	M
18.1	Unknown					soapy	M			
16.00	(16.00) Ethyl hexadecanoate	+	little odor	S	+	little odor	S	+	little odor	S
16.00 to 16.10	Unidentified (m/e 250)		musty	S		stale, chemical	S		jammy, unpleasant	S
16.00 to 16.10	Unidentified (m/e 256)		musty	S		stale, chemical	S		jammy, unpleasant	S
16.1 to 16.4	(16.00) Cinnamyl alcohol	±	pleasant, peppery cinnamon	S	±	spicy, peppery	L	±	peppery	M
16.2 to 16.3	Unknown					chemical	S			

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		RAINBOW FLOWAGE (HEADWATERS)			MOSINEE FLOWAGE (DOWNSTREAM)		LAKE WAUSAU (DOWNSTREAM)		
		PROBABLE COMPOUND (I _E /M.S.)	REGION ODOR FROM CHROMATOGRAPH ³	REL- ATIVE PEAK SIZE ⁴	PRES- ENCE ²	REGION ODOR FROM CHROMATOGRAPH ³	REL- ATIVE PEAK SIZE ⁴	PRES- ENCE ²	REGION ODOR FROM CHROMATOGRAPH ³
16.35 to 16.45	Unidentified (m/e 276)	plant-like	S		black pepper	L		fatty, stale, musty	M
16.4 to 16.5	Unidentified (m/e 258)				black pepper	L		fatty, stale, musty	M
16.4 to 16.6	Unknown	clean musk-like	S		pleasant, sweet	S		sweet	M
16.5 to 16.6	Tricosane	+ no odor	S	+	no odor	S	+	no odor	M
16.5 to 16.6	(16.51) 2,5-Diisopropyl phenol	chemical	VS		cleaning compound, iodine-like	M	+	iodine-like, musty	M
16.6	Unknown				piney	S			
16.6	(16.66) Diethyl phthalate	+ diethyl phthalate- like	L	+		S	+		M
16.7	Unknown	plant-like	S						
16.7	Unknown	burnt hair	VS				-		
16.9	Unknown				heavy chemical	M	-		
18.2	Unknown				cooked vegetables	M		pleasant, plant-like	M
18.3	Unknown						-	rosiney, woody, piney	L
18.3 to 18.5	(18.35) Vanillin	-		+	vanilla-like	M	+	vanilla-like	M

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RETENTION INDEX ¹ (I _E)		SOURCE OF WALLEYED PIKE SAMPLES								
		RAINBOW FLOWAGE (HEADWATERS)			MOSINEE FLOWAGE (DOWNSTREAM)			LAKE WAUSAU (DOWNSTREAM)		
		PROBABLE COMPOUND (I _E /M. S.)	PRESENCE ²	REGION ODOR FROM CHROMATOGRAPH ³	REL- ATIVE PEAK SIZE ⁴	PRESENCE ²	REGION ODOR FROM CHROMATOGRAPH ³	REL- ATIVE PEAK SIZE ⁴	PRESENCE ²	REGION ODOR FROM CHROMATOGRAPH ³
Carbowax 20M EXP. (REF.)										
18.8 to 19.0	(18.90)	Diisobutyl phthalate	-		+			+		
>>20	(>>24)	Dioctyl phthalate	-		+			+		

¹Relative to standard ethyl esters on Carbowax 20 M columns; Experimentally determined from authentic compounds unless otherwise indicated.

²+ Presence indicated by MS and I_E data correlations.

+ Presence indicated by MS, or I_E, and odor associated with the peak during elution from the Carbowax column.

- Presence not indicated in sample by MS, or I_E, and odor.

³Determined by observing the aroma of peaks as they eluted from GC column effluent splitter.

⁴VS = very small; S = small; M = medium; L = large; VL = very large; VVL = very, very large.

^aShibamoto, T.; et al. (50).

^bJohnston, J. C.; et al. (51).

^cPittet, A. O.; Nruze, D. E. (52).

^dKinlin, T. E.; et al. (53).

^eJennings, W.; Shibamoto, T. (54).

^fSwoboda, P.A.T.; Peers, K. E. (55).

^gWolradt, J. P.; et al. (56).

Table II. Characteristics of potential tainting compounds found in walleye pike from the Upper Wisconsin River.

Compound	Carbowax 20 M I _E	Major MS Fragments	General Neat Compound Aroma
Phenol	13.38	<u>94</u> ,66,65,39,40,95,55,47	chemical, medicinal
<u>Methyl Phenols (Cresols)</u>			
o-	13.08	<u>108</u> ,107,79,77,39,90,51,27	chemical, alkyl phenol-like
m-	13.98	<u>108</u> ,107,79,77,91,39,51,53	chemical, alkyl phenol-like
p-	14.11	<u>108</u> ,107,77,79,28,91,109,39	alkyl phenol-like, indole-like
<u>Dimethyl Phenols (Xylenols)</u>			
2,3- ²	14.79	107, <u>122</u> ,121,77,39,79,91,51	-----
2,4-	14.00	<u>122</u> ,107,121,77,39,91,79,51	bad indole, alkyl phenol
2,5-	14.73	<u>122</u> ,107,121,77,39,27	stale, musty
2,6- ²	12.51	<u>122</u> ,107,121,77,39,91,79,51	-----
3,4-	15.20	107, <u>122</u> ,121,39,77,51,27,91	phenolic, medicinal
5,6- ²	14.84	93,39,44,66,28,43,65,92, <u>122</u>	-----
<u>Isopropyl Phenols</u>			
2-	14.00	121, <u>136</u> ,103,91,77,122,51,65	bad indole-like, alkyl phenol-like, medicinal
3-	15.38	121, <u>136</u> ,103,91,77,122,65,71	medicinal, phenolic
4-	15.38	121, <u>136</u> ,103,91,77,122,65,51	medicinal, phenolic
<u>Diisopropyl Phenols</u>			
2,4-	15.53	163, <u>178</u> ,164,121,135,107,103	aromatic-like, heterocyclic
2,5-	16.51	163, <u>178</u> ,164,121,117,91,74,41	chemical, cleaning compound-like, iodine-like
2,6-	13.10	163, <u>178</u> ,91,117,41,39,43,74	phenolic, medicinal
3,5-	16.58	163, <u>178</u> ,135,43,107,164,97,41	chemical, cleaning compound-like
<u>Methyl Isopropyl Phenols</u>			
Thymol [5-methyl-2-(1-methyl ethyl)-phenol]	14.87	135, <u>150</u> ,136,134,91,107,105,55	medicinal, musty
Carvacrol [2-methyl-5-(1-methyl ethyl)-phenol]	15.34	135, <u>150</u> ,107,91,136,105,107	medicinal, phenolic, hot leather
<u>Aromatic Thiols</u>			
Thiophenol	8.72	<u>110</u> ,109,66,84,77,65,51,50	stench, sharp chemical, sulfury

Table II. Contd.

Compound	Carbowax 20 M I _E	Major MS Fragments	General, Neat Compound Aroma
p-Thiocresol	9.75	91, <u>124</u> ,45,39,89,90,123	stench, burnt chemical, rubbery, fruit-like
p-Methoxy- thiophenol	12.41	<u>140</u> ,125,97,139,141,45,41,43	stench, burnt plant

¹Listed in order of decreasing intensity; parent ion underlined.

²Isomers which were not identified in the flavor extracts, but which may be formed.

Figure 1.

Sketch of study area section of the Upper Wisconsin River showing general location of population centers and impassable dams (Number of paper mills: Rhinelander, 1; Merrill, 1; Brokaw, 1; Wausau, 2; and Mosinee, 1).

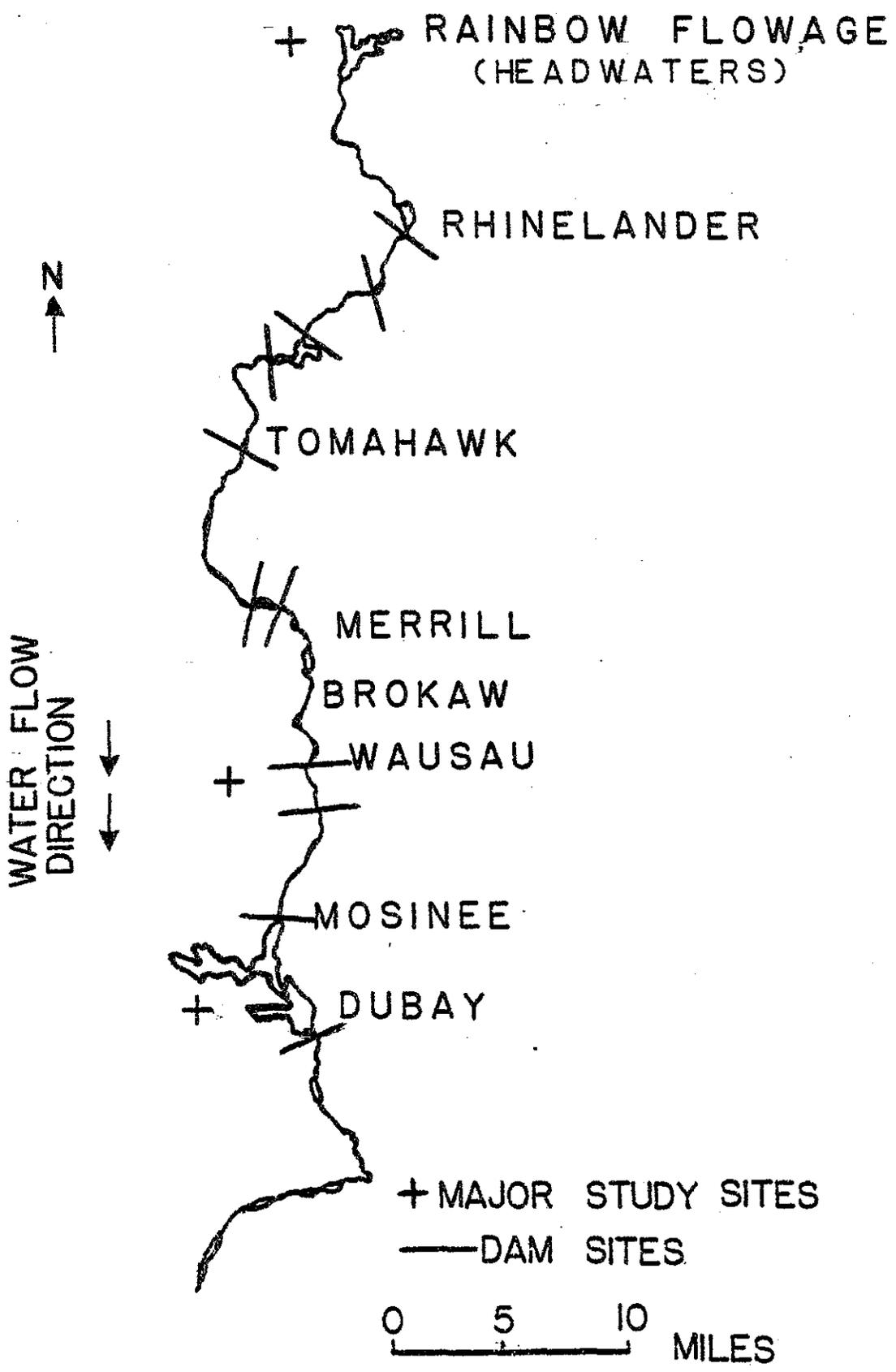


Figure 2.

Carbowax 20 M FID-GC analysis of flavor extract of walleye pike
from Rainbow Flowage (Wisconsin) headwaters site.

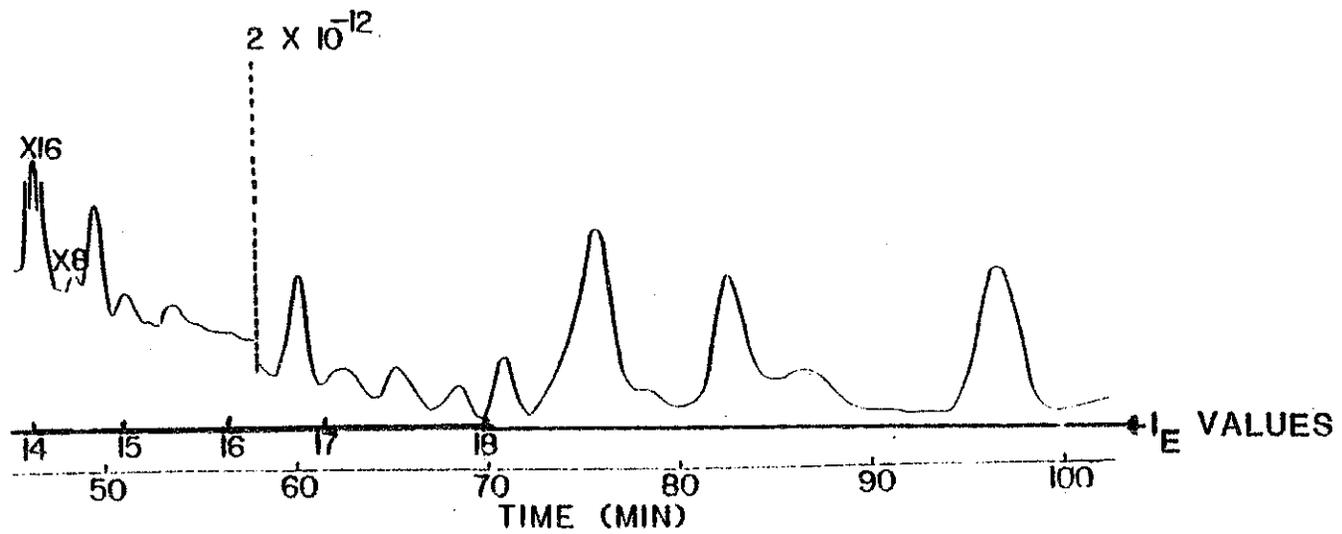
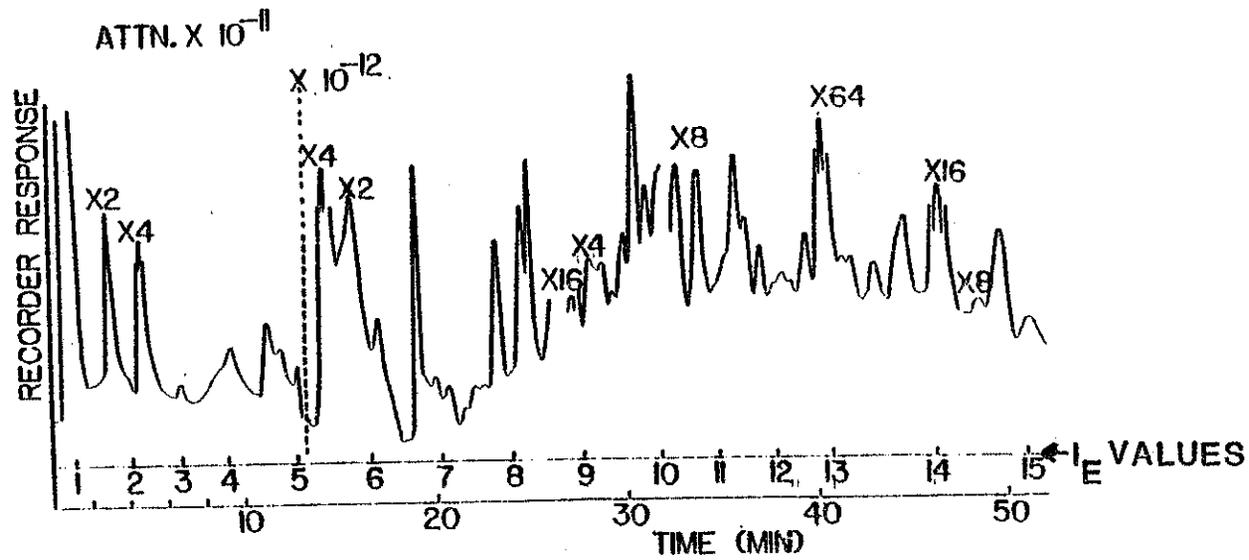


Figure 3.

Carbowax 20 M FID-GC analysis of flavor extract of walleye pike
from Lake Wausau (Wisconsin) downstream site.

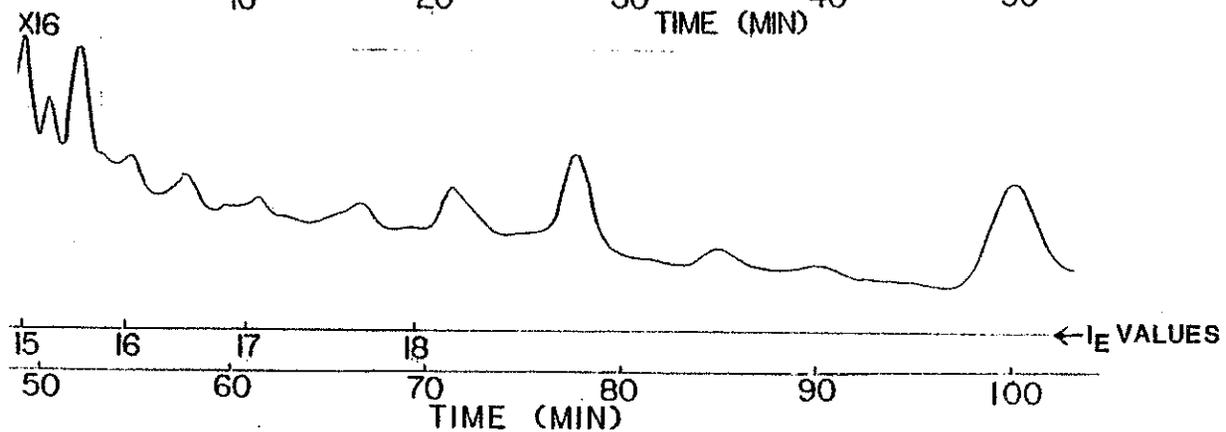
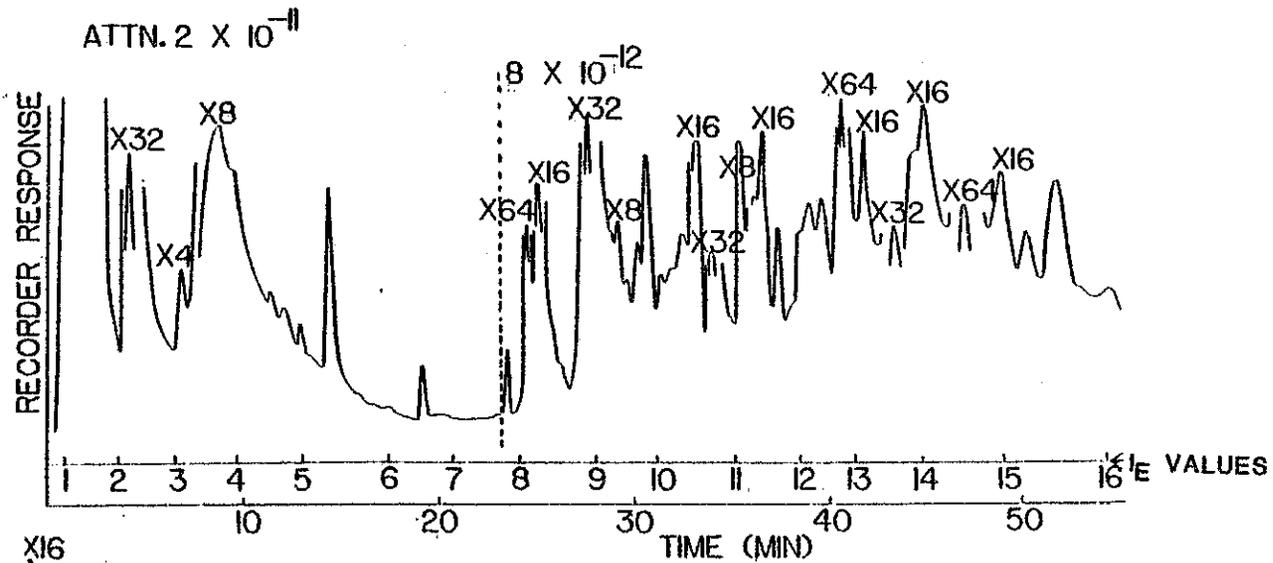


Figure 4.

Carbowax 20 M FID-GC analysis of flavor extract of walleye pike
from Mosinee Flowage (downstream) site.

ATTN. 2×10^{-11}

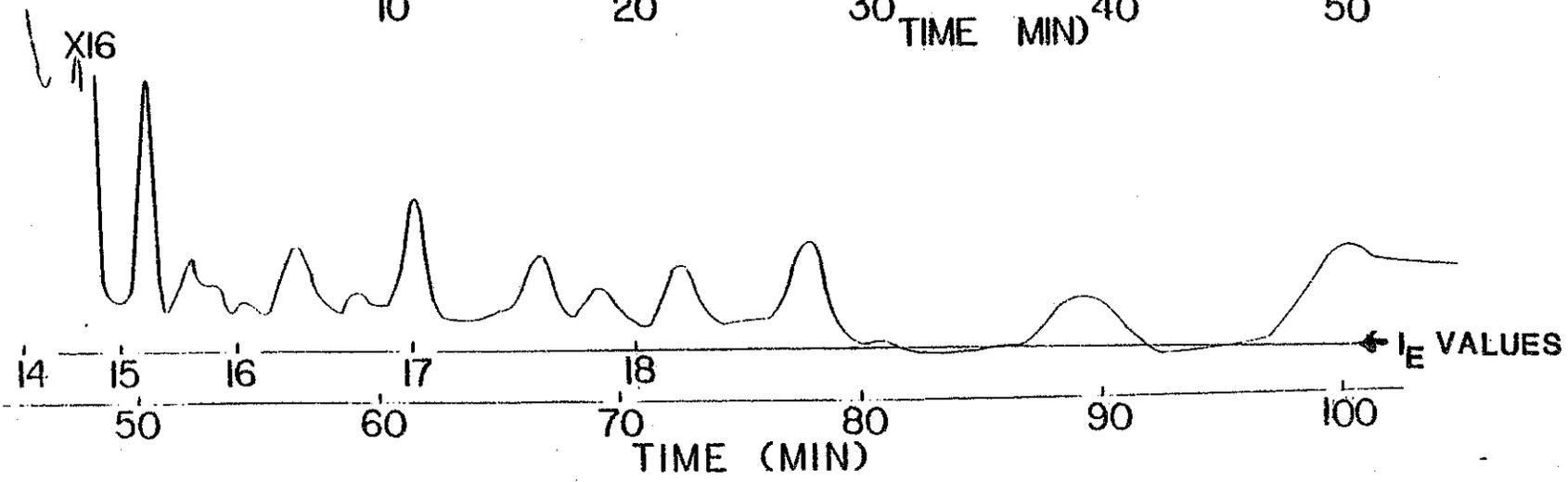
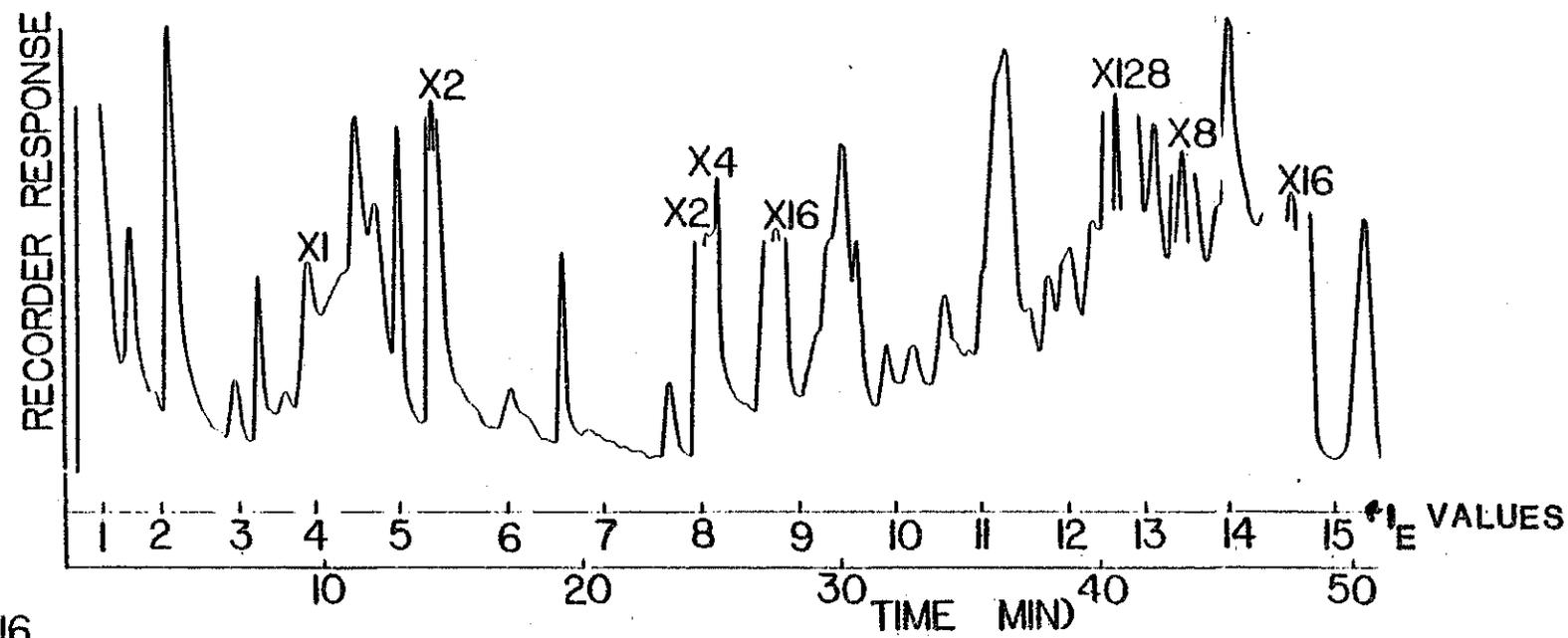


Figure 5.

Carbowax 20 M FPD-GC analysis for sulfur compounds in flavor extract from Mosinee Flowage (Wisconsin) downstream site: Peak No. 1, thiophenol, $I_E = 8.79$; Peak No. 2, p-thiocresol, $I_E = 9.6-9.8$; Peak No. 3, p-methoxythiophenol, $I_E = 12.3-12.4$; Peak No. 4, benzothiazole, $I_E = 13.2-13.4$.

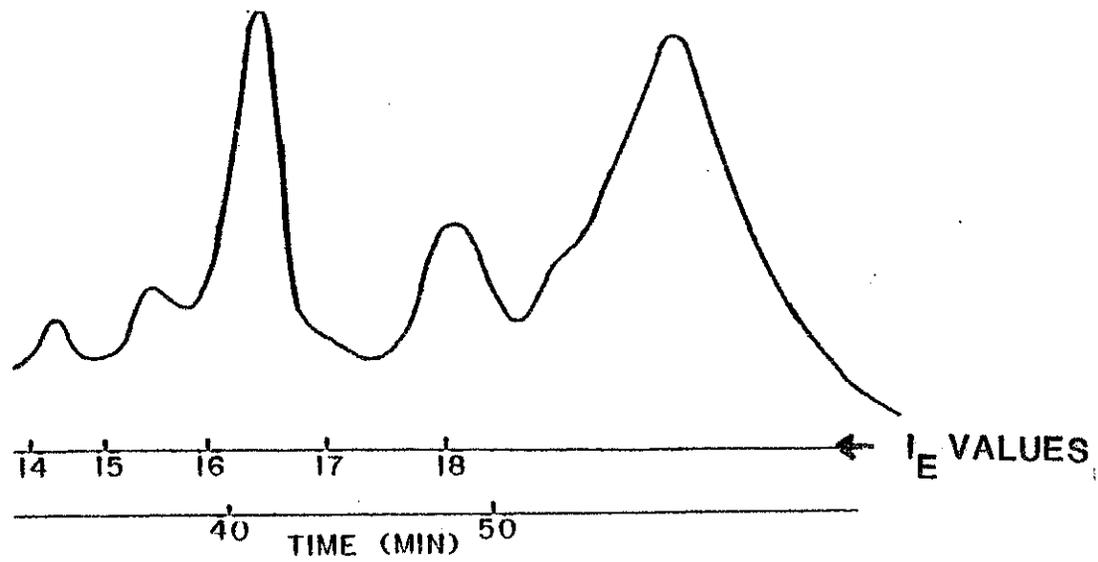
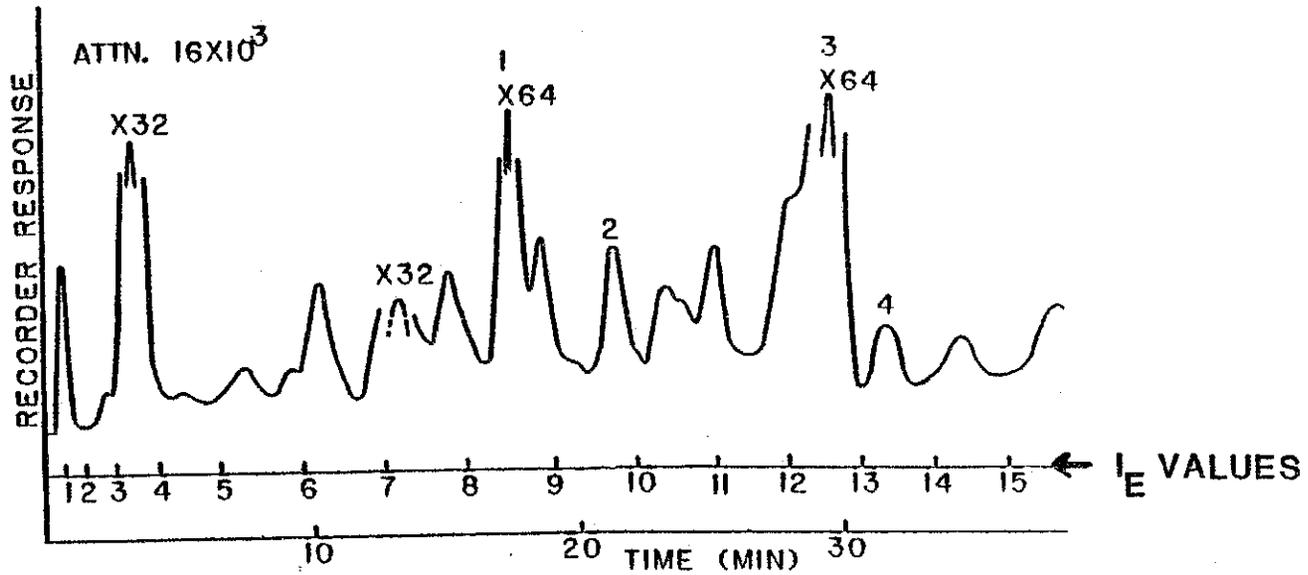


Figure 6.

Mixed SE-30/OV-210 EC-GC analysis of flavor extract of walleye
pike from the Mosinee Flowage (Wisconsin) downstream site.

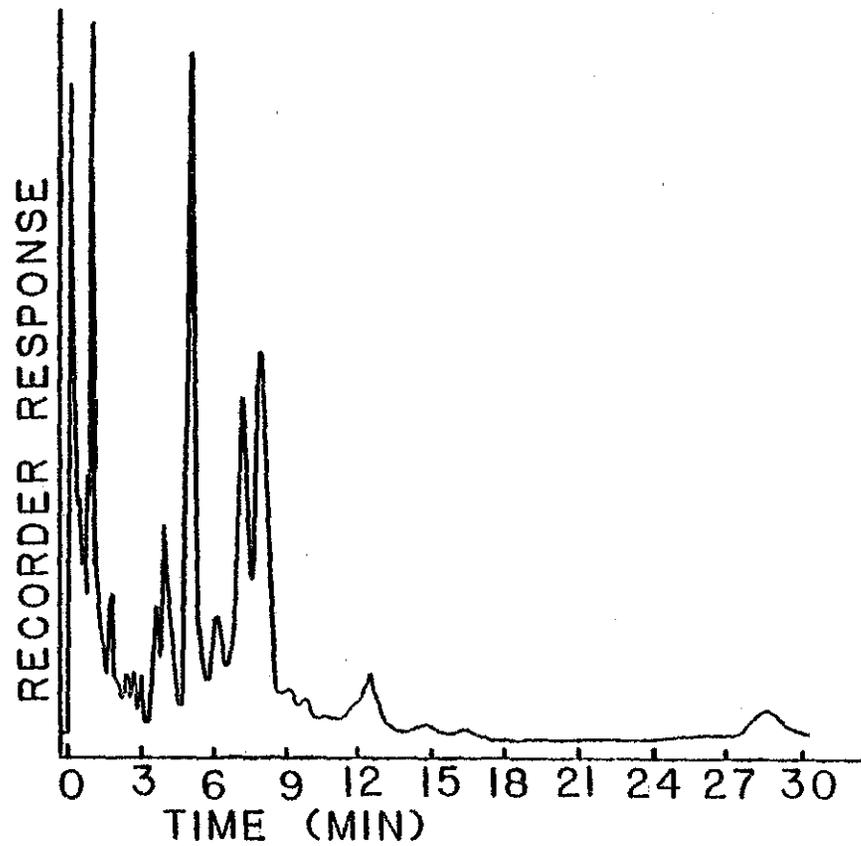
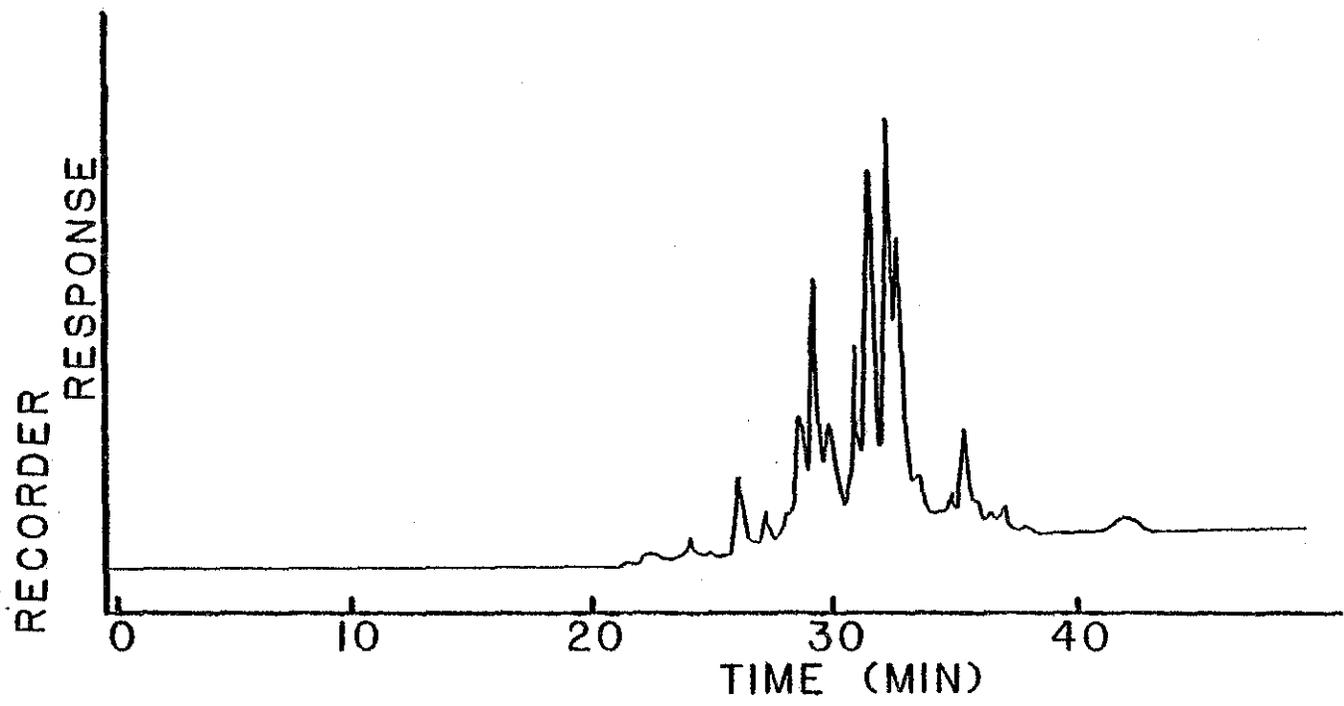


Figure 7.

OV-101 Hall detector-GC analysis of flavor extract of walleye pike
from the Mosinee Flowage (Wisconsin) downstream site.



CHAPTER 3

EVALUATION OF POTENTIAL ROLES OF SELECTED COMPOUNDS IN THE
FLAVOR-TAINING OF UPPER WISCONSIN RIVER FISH¹

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ABSTRACT

Sensory analysis of broiled fish fillets demonstrated that all sports fish in downstreams Lake Wausau and Mosinee Flowage sampling sites on the Upper Wisconsin River were tainted by compounds causing flavors described as phenolic, petroleum, chemical, muddy, and papery. Flavor qualities and thresholds for thiophenol, isopropyl phenols and diisopropyl phenols were determined in both water and cooked fish. Mixtures of thiophenol, 3-isopropyl phenol 2, 4-diisopropyl phenol and carvacrol were shown individually or in mixtures to produce tainting flavors similar to those in wild fish in both direct spiking and controlled fish-exposure studies. Seasonal cycles have been proposed in relation to changes in the availability of alkyl phenols and biologically mediated muddy aroma compounds from diterpene precursors. Suggested pathways for the formation of aromatic thiols from lignin during alkaline pulping have been proposed.

INTRODUCTION

The identification of volatile compounds isolated from flavor-tainted walleye pike (Stizostedion vitreum) obtained from study sites near pulp and paper mills on the Upper Wisconsin River has been discussed in an earlier paper (1). Although the relationship between industrial activities and flavor tainting in fish has received considerable attention (1-15), the identities and roles of offending compounds have remained obscure or unknown, particularly in relation to those associated with pulp and paper processing.

Baldwin et al. (16) studied the flavor of three species of fish from four different sites in Wisconsin including a cold deep water lake, a shallow mixed-water lake, and two flowage sites above and below effluent entry points. Fish from the site below effluent entry, especially walleye pike, was of poorer flavor quality than those from above effluent sources. In this study (14), pulp and paper mill effluents were implicated in the tainting problem because they were the predominant industry along the flowage. Calbert et al. (17) observed that the flavor acceptability of walleye pike collected above industrial and municipal entrance points along the Flambeau River (Wisconsin) were consistently higher than those obtained from downstream sites. They also noted that the common descriptors applied to off-flavors by panelists were chemical, petroleum-like and metallic rather than characteristic terms indicating the sulfury notes associated with paper mills. Tamura et al. (18), Westfall and Ellis (19), Hasselrot (20) and Thomas (21) have all reported tainting of

fish by exposure to pulp and paper mill effluents. Fetterhoff (22) held rainbow trout (Salmo gairdneri) in live boxes located downstream from a variety of industrial effluent outfalls to establish the source of off-flavor in Muskegon Lake (Michigan) fish. They found palatabilities of fish held near the outfalls from both a petroleum refinery and a Kraft pulp and paper mill were significantly lower than control fish.

Tainting has been observed also in rainbow trout exposed to Kraft pulp mill foul condensate 0.015% (V/V) effluent concentration by Leim and Naish (23). Cook et al. (24) observed tainting in yellow perch (Perca flavescens) captured at immediate paper mill outfall areas, but not two to three miles downstream. They also demonstrated that tainting could be caused by Kraft mill condensates and sulfite waste liquors. Farmer et al. (25) observed tainting in yellow perch exposed to evaporator condensate, digester effluent, recovery furnace flue gas condensate, and Kraft mill evaporator condensate, but were unable to demonstrate flavor differences in perch collected from above and below a paper mill discharge on the St. Lawrence River. Whittle and Flood (26) found that when effluent from a mill employing steam stripping to treat digester blow-gas and evaporator condensates were used in tainting studies, a 3% concentration was the lowest level which could be used and still produce flavor impairment of yearling rainbow trout. Shumway and Chadwick (27) reported substantial tainting in salmon (Oncorhynchus kisutch) exposed to 1-2% dilutions (V/V) of untreated Kraft mill effluents. In the same study, salmon exposed to biolog-

ically treated effluent concentrations as high as 2.9% (V/V) exhibited little or no flavor impairment. Farmer et al. (25) reported that 5-10% (V/V) solutions of total Kraft mill effluent concentrations resulted in objectionable flavors in yellow perch. Shumway and Palensky (28) estimated flavor impairment thresholds of 6% (V/V) for Kraft mill effluent and 30% (V/V) for sulfite waste liquor, but reported that no flavor impairment was observed in rainbow trout exposed to biologically stabilized Kraft or sulfite mill effluents at concentrations of 8.1% and 33.7% (V/V), assumed, respectively. Thus, it appears that variations observed in tainting studies reflect differences in processing and effluent treatment conditions between mills and the amount of tainting compounds released into water from Kraft processing.

Shumway and Palensky (28) also exposed rainbow trout, large mouth bass (Micropterus salmoides) and bluegill (Lepomis macrochirus) to many compounds which were considered potentially present in Kraft mill effluents and municipal sewage effluents. They reported tainting flavor threshold concentrations for cresols, mono-, di-, and tri-chlorophenols, ethanethiol, butanthiol, and guaiacol. However, other than the cresols which have been identified in tainted fish recently (1), the flavor properties of specifically-associated tainting compounds have not been previously reported.

The purpose of this paper is to report the characterization of odor and flavor properties of selected alkyl phenols and aromatic thiols that were identified in fish captured below paper mills from

the Upper Wisconsin River (1). Further purposes of this paper are to propose precursors and pathways of formation for alkyl phenols and mud-like aroma compounds from diterpene precursors, and to suggest mechanisms for the formation of aromatic thiols from lignin produced during the alkaline pulping of wood. Finally, earlier observations and reports about the occurrences of flavor tainting of fish in waterways exposed to pulp and paper mill effluents are related to the current findings and means for reducing flavor-tainting in fish are suggested.

MATERIALS AND METHODS

Fish samples

Walleye pike (S. vitreum), northern pike (Esox lucius), black crappie (Pomoxis nigromaculatus), yellow perch (P. flavescens), and bullhead (Octalurus sp.) were collected from the Wisconsin River by fyke nets or electroshocking by the State of Wisconsin Department of Natural Resources from a study site in the Rainbow Flowage headwaters and at downstream study sites in Lake Wausau and the Mosinee Flowage (1). These fish were captured in the early summer (June-July) of 1979 to determine the extent and type of tainting flavors present in sports fish populations located below paper mills.

All fish were iced immediately upon capture, and were filleted, skinned, packaged, and frozen in a manner similar to that described earlier (1). No attempt was made to segregate fish according to size, but all were considered harvestable by the sport fishery on that species. These fish were used to document across-species flavor characteristics and sampling-site influences on flavors. Walleye pike and northern pike were used additionally for studies employing spiking of suspected flavor-tainting compounds. Similarly collected walleye pike obtained in 1980 from additional headwaters and downstream sites in late spring (May-June) and late summer (August) were used in a limited evaluation of seasonal effects on flavor tainting in the study areas.

Commercially-obtained whitefish (Coregonus clupeaformis) and cod fillets (Gadus morhua) were also used in screening evaluations of

suspected flavor-tainting compounds in spiking studies. Aquacultured yellow perch (P. flavescens) (courtesy of the University of Wisconsin Sea Grant Institute Aquaculture Laboratory) were used in paper mill foul condensate exposure studies, and similarly aquacultured rainbow trout (S. gaidneri) were employed in exposure studies involving selected combinations of flavor-tainting compounds.

Paper mill foul condensate and tainting compound exposure studies were carried out in glass tanks (163 L) filled with fresh well water. Foul condensate was added directly to the tanks at levels of 0.1% and 0.2% by volume to separate tanks, and 10 perch (180 g each) were placed in each tank. Tainting compounds were dissolved in 10 ml quantities of ethanol before introduction into exposure tanks, and after thorough mixing, 6 trout (ca 1 kg each) were placed in each tank. Perch were maintained at 22°C and trout were held at 12-15°C. Dissolved oxygen was maintained by aeration at 9-11 ppm, and water was not added or exchanged during the exposure periods. All fish were exposed to experimental environments for 24 hrs before processing into skinned fillets for sensory evaluations.

Preparation of samples for sensory analysis

If required, skinless fillets (except for trout) were thawed in cool water, drained, and then placed on a broiler pan for preparation as mildly seasoned, broiled fillets. This involved lightly brushing with melted butter, sprinkling lightly with salt and ground black pepper, and broiling in a conventional electric household-type oven for five to ten min, or until done as indicated by easily flaking with a fork.

Entire fillets were then carefully flaked, and those within a lot were uniformly mixed before dispensing or further treatment. Samples were covered with aluminum foil to prevent moisture losses, and then were kept warm (50-60°C) under infrared lamps.

For direct assessments of flavors, flaked fish was dispensed directly to panelists. Where less intense flavors were desired, weighed portions of flaked, tainted fish were mixed with appropriate amounts of non-tainted fish. Spiking studies were carried out by adding the appropriate concentration of tainting compounds to flaked fish from aqueous stock solutions prepared by first dispersing compounds in 95% ethanol. The concentration of ethanol in flaked fish was kept below the threshold (10 ppm; 29), and samples were thoroughly mixed and equilibrated for about 15 min before assessment.

Selected compounds were evaluated for odor and flavor quality and detection thresholds at 21°C in aqueous solutions prepared from odor-free, membrane-deionized water. Samples were prepared from stock solutions of compounds in 95% ethanol.

Sensory analysis procedures and conditions

Sensory analyses were performed using facilities and procedures generally accepted for the sensory evaluations of foods (30, 31). Initial evaluations and screening of compounds for flavor characteristics and suitable concentrations were carried out under conditions of free communication by two to three individuals experienced in flavor characterizations. Panel evaluations were conducted in individual panelist booths equipped with running tap water, and the

room was illuminated with indoor fluorescent lighting (78 fc).

Each panelist was given samples of about 60 g of each fish in odor-free, clear plastic 150 ml cups which were coded with random 3-digit numbers. Fish samples were served at approximately 45°C, and water samples were served at room temperature (ca 21°C).

A ballot containing a semi-structured linear scale for generalized off-flavor intensity (none to extremely pronounced) was employed for the quantitative descriptive analysis (32, 33) of fish samples. A similarly structured preference scale (dislike extremely to like extremely) was also included on this ballot to indicate the relationship between intensity of off-flavors perceived and palatability of fish. Ballots were coded on a seven point basis, and data from each panel evaluation were analyzed for differences between samples using analysis of variance suitable for a randomized block design (34).

Flavor thresholds for compounds were determined for the 50% response level described by Patton *et al.* (35) using the multiple sample procedure reported by Siek *et al.* (36).

A special ballot based on overall degree of difference measurement principles (37) was designed for the simultaneous determination of similarities in qualities and intensities of tainted flavors in fish samples. Panelists were served an identified reference sample (coded R), and were asked to compare each coded sample served to the reference sample. Responses were recorded on a structured, continuous similarity scale (see Table III for descriptors), and these data were coded on a seven point basis for statistical analysis as

described earlier for flavor intensity and overall preference determination.

Panels were composed of 28 to 30 members who were experienced in the sensory analysis of foods, including fish. Rigorous training of panelists for specific flavors was not practiced, and since the preparation of fish samples simulated usual cooking, it was assumed that panel results reasonably reflect those expected from the general population.

RESULTS AND DISCUSSION

Descriptive sensory analyses were conducted for several fish species from the three major study sites during the 1979 sampling periods to define the extent of flavor impairment, and to obtain a set of descriptors for the tainting flavors observed. The results of the panels (Table I) confirmed that all sports fish in the downstream study areas of Lake Wausau and the Mosinee Flowage exhibited similar and pronounced degrees of flavor impairment compared to the headwaters control fish from Rainbow Flowage. Each study site was separated from others by impassable dams which prevented mingling of populations of fish from study sites, and similarities noted in the quality of flavors causing flavor-impairment indicated that offending compounds were distributed in the entire downstream study section. The feeding habits of fish included in the evaluations varied widely, and since all species were similarly affected, a particular diet could not be related to an accumulation of causative agents for flavor-tainting.

Voluntary descriptive comments from taste panelists along with those of more experienced flavor assessors were found to frequently parallel gas chromatographic peak odors observed and reported previously (1). Medicinal, phenolic, chemical-like and petroleum-like descriptors were often used to describe both fish flavors and GC effluent odors. GC-MS analysis of compounds in the chromatographic regions with these odors confirmed the presence of many alkyl phenols which exhibit these characteristics. Similarly, muddy, musty and earthy flavors observed by panelists were noted in the GC effluents,

and the principal ones were identified by GC-MS as geosmin and 2-methyl-isoborneol.

Papery, pulp mill, cardboard-like descriptors were used by panelists, and apparently reflected combination flavor effects of aromatic thiols and alkyl phenols. While extensive studies were not carried out on the flavor qualities of alkyl phenols and aromatic thiols, many bench-level evaluations of individual compounds were made, and these are summarized in Table II. It should be borne in mind that the odor and flavor characters of odorants and flavorants depend upon the concentration and the system evaluated (41, 42). Thiophenol gave rich, meaty flavor sensations in fish at 10 ppb, but at 20 ppb the sensation was that of a combination of an unusual burning, old fish oil, stale, papery and rubbery notes. Carvacrol and 2-isopropyl phenol gave slightly metallic and chemical flavors to fish. Petroleum-like flavors were produced at some concentrations by 2-isopropyl phenol, 3-isopropyl phenol, 2,4- and 2,5-diisopropyl phenols in water. The same flavor descriptor, petroleum-like, was applied to carvacrol, 3-isopropyl phenol, and 2,4-diisopropyl phenol in fish. Flavor notes indicative of the paper-type were produced by thiophenol, 2-isopropyl phenol and 3-isopropyl phenol. Licorice-like flavors were unexpectedly observed for solutions of 250 ppb 2-isopropyl phenol, and one and five ppb of 2,4-diisopropyl phenol in water, but these notes were not expressed in fish media.

Based on similarities with flavors noted in tainted wild fish, carvacrol, 3-isopropyl phenol and 2,4-diisopropyl phenol were selected

as highly influential flavor-tainting compounds for further study.

Each had a flavor or odor characteristic similar to those observed in downstream tainted fish, and each was also found to have low threshold values in both water and fish systems which indicated that accumulation of these compounds could easily result in suprathreshold levels in fish. When 100 ppb each carvacrol, 3-isopropyl phenol and 2,4-diisopropyl phenol were added to fish, a papery, wet-wool yet sharp phenolic flavor was observed. Changing only the 2,4-diisopropyl phenol concentration in the mixture to 250 ppb caused the flavor to become green, medicinal, papery, wet-wool, petroleum-like, and gave the sensation of strong lingering aftertaste flavors which were similar to those observed in the flavor-tainting present in fish from the Upper Wisconsin River.

Hydrogen sulfide, methyl mercaptan, dimethyl sulfide and dimethyl disulfide are the four main organic sulfur constituents that are found in Kraft mill odor emissions (43). Although thiophenol has not been reported to be a component of pulp mill odor, it has an unpleasant odor, and was identified in extracts of tainted fish. This compound was found to have a recognition threshold of about 15 ppb in fish (Table II). A combination of 50 ppb of 3-isopropyl phenol and 10 ppb of thiophenol in fish gave a sharp papery, cardboardy flavor similar to the flavor of tainted northern pike. A combination of 50 ppb carvacrol, 3-isopropyl phenol and 2,4-diisopropyl phenol plus 10 ppb thiophenol was not as objectionable as the 3-isopropyl phenol and thiophenol combination. However, these combinations also appeared to be very similar to the tainting-flavors found in fish from the Upper

Wisconsin River. When thiophenol was added to fish preparations containing phenolic compounds, it was observed that it acted as a blender which ameliorated harsh, medicinal and phenolic notes, and resulted in flavors suggestive of wet paper notes mentioned earlier for flavor-tainted wild fish.

The results of a taste panel that evaluated the flavor effects of the four selected compounds added to broiled, minced, untainted northern pike are shown in Table III. In this evaluation panelists were asked to compare the spiked samples with samples of wild tainted northern pike from the Mosinee Flowage downstream site. Most panelists perceived fish spiked with both formulations to be qualitatively similar to the coded reference, an environmentally tainted northern pike sample. Interpretation of these data are strongly influenced by panelist reactions to the coded Mosinee northern pike samples which contained typical tainted flavors of fish from the study area. The frequency of panelist responses for this sample was similar to those for the spiked samples. Several of the panelists were unable to clearly associate all of the flavors in the samples, and this is interpreted as due to carry-over flavors among the samples as well as individual insensitivities to some compounds. Sensitivities to flavor compounds and combined effects of compounds for individual panel members would be expected to be a factor in the recognition of tainting-flavors. Anosmia or insensitivity to given compounds (44), and a number of psychological factors (30) may influence individual panelists. Most important, however, is the large number of panelists

(18-19 of 30) who expressed a perception of distinctly similar qualities and intensities of tainting flavors for each of the samples without any prior training or panel member selection. Thus, these sensory data strongly support the analytical chemical data obtained earlier (1) in the establishment of a cause-effect relationship in fish for the flavor tainting compounds.

In order to demonstrate the accumulation of implicated compounds, rainbow trout were exposed to static concentrations of simulated tainted mixtures for 24 hrs. Preliminary tasting of the exposed trout by experienced panelists revealed that these fish were too strongly tainted for presentation to panel members. Thus, dilution series were prepared with minced, cooked fish of each of the tainted fish groups and control fish flesh, and samples with concentrations most closely simulating naturally tainted fish were selected for serving to panelists. Trout flesh was quite different from northern pike flesh so panelists were asked only to compare the flavor of the reference northern to each coded sample of trout, and a coded reference was not included. Overall data in Table IV show that overall panelists perceived all of the trout samples to exhibit characteristics similar to the naturally tainted Mosinee Flowage northern pike reference sample. Voluntary comments included the terms phenolic, medicinal, chemical, metallic, gasoline-like, fishy, rubbery and sulfury. Many of these were the same as those developed during preliminary flavor screenings discussed earlier. It is important to note that trout containing the single tainting compounds, 3-isopropyl phenol (tank No. 1) and thio-

phenol (tank No. 2), were perceived as very similar in type and intensity to the naturally tainted northern pike. The more complex mixtures (tank Nos. 3 and 4) elicited slightly less unanimity among panelists overall, but these samples were perceived by several as much stronger than the reference tainted northern pike. The high flavor strengths in these samples probably confused even experienced panelists, and caused the higher numbers of responses in the definitely not the same flavor-type category. However, overall these data are interpreted as confirmatory to the proposed role for alkyl phenols and aromatic thiols in the flavor-tainting of fish in the Upper Wisconsin River.

An attempt was made also to simulate the tainting found in Wisconsin River fish by exposing yellow perch to foul condensate obtained from a paper mill. Aquacultured perch were exposed to water containing 0.1% or 0.2% (V/V) of foul condensate for 24 hrs. Sensory analysis of these fish (Table V) showed that only modest off-flavors were noted, and the preference scores were not significantly lowered. Although the foul condensate sample as obtained had an objectionable, penetrating odor, the odor apparently was caused by more volatile and less influential flavor-tainting compounds than thiophenol and alkyl phenols found in tainted wild fish.

Further sensory evaluations of walleye pike conducted in 1980 showed a strong seasonal effect on off-flavor intensities (Table VI). The flavor characteristics of spring caught fish replicated the overall pattern observed for both headwater and downstream walleye pike tested

in 1979. Voluntary comments of panelists indicated that similar flavors, i.e., chemical, medicinal, aromatic, and petroleum-like dominated in these fish. However, a fall sampling of walleye pike gave average mean off-flavor intensity scores for fish from headwaters and downstream sites that were not significantly different from each other (Table VI). The headwaters fish had become even more off-flavored than those from the downstream sites, and the dominant flavors for both sites were muddy, musty and earthy. However, there was still a recognition of chemical, medicinal flavors in the downstream fish although the intensities of these were much less than that observed in spring-captured fish.

Semi-quantitative comparisons of GC patterns from walleye pike collected in 1979 also indicated that levels of alkyl phenols were greatly affected by relatively short seasonal changes. Representative GC patterns for the $I_E = 15.25$ region in Carbowax 20 M separations, an area with a phenolic, medicinal and chemical aromas attributed to 3- and 4-isopropyl phenols, 3,4-dimethyl phenol and carvacrol are shown in Figure 1. Aromas associated with this region changed from strong medicinal, phenolic for samples collected early in the summer at stream sites to downstream sites to a weak, hot plastic, burnt aroma for samples collected in July from the Mosinee Flowage. A distinct odor was not associated with this region in the Rainbow Flowage walleye extract sample. Thus, these analytical data support the seasonal variation in flavors noted by sensory analysis, and strongly suggest a cyclic occurrence of alkyl phenols in the river.

Thymol and carvacrol have been reported to be present in the essential oils of many plants (45). Cresol, ethyl, phenol, thymol, and carvacrol have been reported also in licorice essential oil (46). Diisopropyl phenols were recently reported in Spanish origanum oil (47). Steinke and Lindsay (48) have reported the identification of isopropyl phenol isomers in Coho salmon obtained from Lake Michigan, as well as in sediments from Mud Lake (Wisconsin) which is essentially free from industrial or municipal activity. Nishimure and Masuda (49) isolated *p*-isopropyl phenol and other alkyl phenols as minor constituents of whiskey oils. In a review of phenols and phenolic compounds in food, Maga (50) cites many references for the occurrence of phenols in plant-derived food products and wood smoke.

Structurally, terpenes offer very attractive possibilities as precursors of methyl and isopropyl phenols. Terpenes are biosynthesized through the generalized scheme shown in Figure 2. For example, *p*-cymene and thymol could be derived directly from geranyl pyrophosphate. *p*-Cymene, which was identified in walleye flavor extracts, theoretically also could be hydroxylated to yield carvacrol, but this seems unlikely at this time. The multiple methyl and isopropyl groupings observed for several phenols identified in the fish extracts indicate a more complex precursor system than a monoterpene-like *p*-cymene.

A unifying hypothesis to explain the presence of the variety of isopropyl-substituted phenols found to cause fish tainting requires a key or focal intermediate compound such as is shown in Figure 3 (A).

Naturally occurring compounds with alkyl structures similar to the key intermediate have been found by Laflamme and Hites (51), and are shown in Figure 3 (B and C). These homologues were identified in Yosemite soil extracts, and are considered naturally occurring intermediates of environmental degradation of original rosin constituents since neither have been reported to be present in pine rosin (51). These compounds illustrate important structures in relation to the proposed key intermediate, and these are the open center ring of (B) and the methyl group on (C) instead of the carboxyl group found on the resin acids. The resin acids are shown to illustrate the structural similarities of these compounds to the key intermediate. Abietic acid is an isomerization product of levopimaric and neoabietic acids, and is almost absent from fresh samples of rosin from pine trees (52).

A unified scheme for the formation of alkyl phenols and biologically mediated muddy, musty, earth-like aroma compounds is shown in Figure 4. Mechanisms of formation of these compounds from the key intermediate, or GGPP, via rearrangements, ring closures and fragmentations are indicated in Table VII. Positional isomers of alkyl phenols found would be expected to occur as the result of intermediates that would allow OH additions at the various sites on the ring. While other terpenes could also serve as precursors for the formation of these compounds, the model diterpene compounds allow illustration of possible means for formation of all compounds in this group implicated thus far in fish tainting.

The cyclic seasonal changes in tainting flavors in the Wisconsin River fish also can be related to alkyl phenols and earthy-musty compounds through diterpene precursors. Sheldon and Hites (53) and Polisois et al. (54) have reported that levels of phenols and partially characterized alkyl phenols in waterways were much higher during winter than during summer months. Additionally, Polisois et al. (54) has reported that the degradation of phenolic compounds is temperature, pH, oxygen concentration, and aquatic biota dependent. Pronounced chemical, phenolic, and medicinal flavors found in fish from the Upper Wisconsin River fish during spring and early summer, 1979, would be the result of accumulations of alkyl phenols either through their formation in paper mill effluents directly or after discharge in the river. Under high ambient temperatures resin acid diterpenes, and presumably the key precursor compound, would be changed to highly water soluble, non-odorous degradation compounds through oxidative reactions (55, 56, 57) and biological processes (58, 59, 60). Under conditions of cold temperatures, reduced river flow and low lighting levels, the volatility of organic compounds, and especially the rate of microbial degradations would be reduced and allow the alkyl phenols to increase in concentration and bioaccumulate in the fish. It is not known at this time whether photocatalytic processes play a role in the proposed diterpene degradations. However, terpenes are quite susceptible to oxidations, and the blue haze typically found in forested areas on hot summer days is caused by photochemical reactions between terpene hydrocarbons and ozone (61).

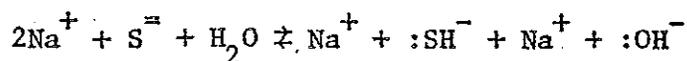
Conversely, late summer and early fall conditions favor the formation of earthy, muddy, musty type flavor compounds which dominate over phenolic tainting flavor compounds because they allow abundant growth of Streptomycetes and algae. To date the formation of geosmin and methylisoborneol has been attributed to total biosynthesis by streptomycetes and algae (62), and the conversion of a precursor from plant sources has not been suggested. However, literature on the flavor constituents of familiar root crops provide strong associative evidence that supports the hypothesis for the existence of a precursor system. The flavor of raw carrots (Daucus carota) depends principally upon mono- and sesquiterpenoid compounds (63, 64, 65), and they usually do not exhibit distinct earthy flavors. On the other hand, red garden beets (Beta vulgaris) exhibit strong earthy, muddy flavors that are due to the presence of geosmin (67, 68), and do not contain abundant mono- and sesquiterpene compounds (68). Thus, it appears reasonable to speculate that flavor differences between the two root crops depends in part on the characteristics of terpene biosynthesis in each. The availability of a suitable diterpene precursor in beets could lead to an enhanced formation of geosmin by synergistically-associated streptomycetes. This ability would not, however, preclude total biosynthetic capabilities for the earthy compounds also by algae and streptomycetes. Finally, these associations could relate significantly to the total diterpene precursor load from mill effluents as coniferous tree processing would also be expected to yield less total diterpenes than hardwood processing.

Phenol, guaiacol, syringol, vanillin, and acetovanillone are the major non-chlorinated phenols found in pulp mill effluents (69, 70, 71) and these compounds are all associated with the lignin fraction of wood. A very generalized scheme showing the relationship of some lignin related compounds is shown in Figure 5. A mechanism for the formation of isopropyl phenol from lignin is included, and this would require an intermediate compound containing a propane ring to be formed. However, evidence for a lignin involvement in the formation of alkyl phenols is virtually nonexistent at this time.

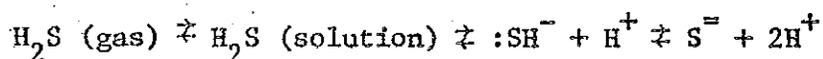
While the evidence for direct involvement of lignin derived phenols in fish tainting in the Wisconsin River is not strong at this time, phenol was found in relatively abundant concentrations in some fish. Damyaneko (72) demonstrated that fish living in water containing small quantities of phenol develop tarry, phenolic flavors, and Westfall and Ellis (19) have confirmed his results. In the current study, attempts to characteristically taint perch via exposure to guaiacol were unsuccessful, and only mild, slightly smokey flavors were imparted. The phenols may, however, serve as possible direct precursors for aromatic thiols, although this reaction mechanism may not be the most favored among those options that are available.

Formation of the aromatic thiols observed through minor, little known side reactions occurring during pulping appears as an attractive option. During Kraft processing, wood chips are cooked at high temperatures in a white liquor or an aqueous solution of sodium hydroxide, sodium sulfide, and inorganic ions including carbonate

($\text{CO}_3^{=}$), sulfide ($\text{S}^{=}$), sulfate ($\text{SO}_4^{=}$), sulfite ($\text{SO}_3^{=}$), and thiosulfate ($\text{S}_2\text{O}_3^{=}$). Hydrosulfide ions ($:\text{SH}^-$) can be derived through two processes. Sodium sulfide may react with water:



or hydrogen sulfide which is always present to some extent in the gas phase of the sulfide solutions may redissolve in solution:



During the pulping process, sulfide, hydrosulfide, and hydroxyl ions ($\text{S}^{=}$, $:\text{SH}^-$, and OH^- , respectively) promote lignin degradation, and inhibit undesirable condensation reactions by competing with carbanions formed during the processing for available reactive sites. Mechanisms for sulfonation of lignin polymers are somewhat unclear although the formation of lignosulfates by the action of sulfide and free sulfurous acid is recognized (73). Sulfur is generally shown bonded at the beta position on the phenyl propane side chain, however, the form of the sulfur present in isolated Kraft lignins varies. Evidence for sulfoxide and sulfone linkages has been reported for Kraft pine lignin (74). However, in more recent studies of Kraft Douglas fir lignin, products such as H_2S and mercaptans that are indicative of thiols and polysulfide functionalities have been isolated (75). These reactions have been discussed because relatively complex sulfonated lignin fragments may provide suitable precursors for aromatic thiols.

Some proposed generalized reaction schemes for the formation of thiophenol through minor side reactions are shown in Figure 6. The

formation of a sulfate ester to serve as a leaving group which should be displacable by the nucleophilic :SH^- group is the most favorable mechanism suggested (reaction 1). The formation of phenolic esters of alkyl fatty acids which are abundant in tall oil (76) could also provide a reasonably good leaving group (reaction 2). A reaction involving the cleavage of a native ether bond could also provide a suitable leaving group, especially after sulfonation of hydroxyl groups (reaction 3). Direct substitution of the :SH^- group for the OH^- group on the aromatic ring is shown in route 4, but for this to occur, formation of phenol along with a high concentration of :SH^- in the white liquor would appear to be required. Phenol is a well documented product of lignin degradation (69, 70, 71). Direct substitution of the :SH^- of :OH^- in alkyl alcohols has been discussed (77), and trace amounts of thiophenols have been observed in cooked beef (78). The phenol precursor for thiophenol in cooked beef may be tyrosine, and :SH^- would probably be derived principally from cysteine.

p-Methoxythiophenol and p-thiocresol could also be formed from similar minor side reactions involving cresol and guaiacol-type precursors in lignin. In the case of p-methoxythiophenol it would be essential to involve an intermediate that would allow reorientation of the -SH group to the p-position from o-position seen for native phenolic precursors. However, the relative abundance of individual aromatic thiols may relate to the amount of each phenol-type precursor available. Because of high flavor strength, total production of

aromatic thiols need not be great to yield adequate amounts to contribute to flavor-tainting. It should also be noted that the aromatic thiols would be oxidized easily to various disulfides which could serve as reservoirs in more complex systems.

Tainting-flavors and compounds responsible for the strongest tainting-flavors observed in walleye pike from the Upper Wisconsin River have been characterized, and terpene and lignin precursors have been proposed. Combinations of chemical and natural biological processes that are accelerated by the availability of greater amounts of precursor materials and favorable environmental conditions appear to be influential in the variable nature of the tainting observed.

Methods for reduction of tainting compounds in fish appear to be available. These include efficient biological reduction of diterpene resin acids and other diterpenes to remove precursors for alkyl phenols, and hydrogen peroxide or potassium permanganate oxidation of aromatic thiols and phenols (79, 80) before they enter the river, especially during winter periods.

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Table I. Descriptive sensory evaluation of fish captured in the early summer of 1979 from the Upper Wisconsin River.

Sample description	Sample attribute	
	Off-flavor intensity ¹	Overall preference ²
	(- - - - Mean Scores ³ - - - -)	
Walleye pike		
Rainbow Flowage	2.76 ^a	4.37 ^a
Lake Wausau	5.50 ^b	2.30 ^b
Rainbow Flowage	2.81 ^a	4.67 ^a
Mosinee Flowage	4.27 ^b	3.25 ^b
Northern pike		
Rainbow Flowage	2.79 ^a	4.39 ^a
Lake Wausau	4.62 ^b	2.91 ^b
Rainbow Flowage	2.78 ^a	4.64 ^a
Mosinee Flowage	4.66 ^b	2.77 ^b
Black crappie		
Rainbow Flowage	2.34 ^a	5.02 ^a
Lake Wausau	5.19 ^b	2.25 ^b
Rainbow Flowage	2.71 ^a	4.66 ^a
Mosinee Flowage	4.86 ^b	2.53 ^b
Yellow perch		
Rainbow Flowage	2.43 ^a	4.83 ^a
Lake Wausau	4.31 ^b	3.13 ^b
Bullhead		
Rainbow Flowage	3.06 ^a	3.95 ^a
Lake Wausau	4.94 ^b	2.46 ^b
Rainbow Flowage	3.60 ^a	3.63 ^a
Mosinee Flowage	5.15 ^b	2.25 ^b

¹Scale: 1 = none; 7 = extremely pronounced.

²Scale: 1 = dislike extremely; 7 = like extremely.

³n = 29±2.

a,b, Mean scores in the same column within a comparison pair with the same superscript are not significantly different at the 0.1% level. Comparison valid between members of pairs only.

Table II. Flavor characteristics of alkyl phenols and aromatic thiols¹.

Compound	Detection Threshold	Odor and Flavor Characteristics
<u>Phenol</u>	0.047 ppm in air (odor) ² 5.9 ppm in water (odor) ² 0.25 ppm in water (taste) ²	Neat: medicinal, smokey, shoe polish-like
<u>Cresols (methyl phenols)</u>		
Cresol	70 ppb in trout ³	
m-Cresol	68 ppb in water ² 200 ppb in trout ³	Neat: phenol-like, fresh hot asphalt-like, wood preservative- like, smokey
o-Cresol	90 ppb in water ² 260 ppb in water ⁴ 65 ppb in water ⁵	Neat: phenol-like, indole-like
p-Cresol	1 ppb in air ⁵ 550 ppb in water ⁴ 120 ppb in trout ³	Neat: phenol-like, fresh hot asphalt-like, wood preservative compound-like, indole-like
<u>Xylenols (dimethyl phenols)</u>		
2,3-Dimethyl phenol	-	Neat: chemical, phenolic; stale, musty in low ppm in air
2,4-Dimethyl phenol	-	Neat: chemical, phenolic; stale, musty in low ppm in air
2,5-Dimethyl phenol	-	Neat: chemical, phenolic
3,4-Dimethyl phenol	-	Neat: chemical, phenolic; like indole in low ppm in air
<u>Isopropyl phenols</u>		
2-Isopropyl phenol	1 ppb in water 2,000 ppb in fish	Neat: phenolic, Lysol-like, medicinal In water: 100 ppb, petroleum-like

Table II continued

Compound	Detection Threshold	Odor and Flavor Characteristics
<u>Isopropyl phenols (continued)</u>		
		250 ppb medicinal, chemical, musty, anise-licorice-like
		In fish: 2,000 ppb astringent, metallic, chemical, green-plant aftertaste 100 ppb sharp papery, oily
3-Isopropyl phenol	5 ppb in water 100 ppb in fish	Neat: phenolic, medicinal In water: 0.5 ppb slight, petroleum-like, 5.0 ppb petroleum-like, chemical
		In fish: 10 ppb papery, heavy, sharp 100 ppb sharp chemical, papery, petroleum-like
4-Isopropyl phenol	-	Neat: phenolic, medicinal
<u>Diisopropyl phenols</u>		
2,4-Diisopropyl phenol	0.91 ppb in water 100 ppb in fish	Neat: phenolic, medicinal; naphthalic, heterocyclic-like in low ppb in air
		In water: 0.5 ppb hydrocarbon-like, oily, gasoline 1 ppb licorice-like, sharp, phenol-like 5 ppb strong licorice
		In fish: 10 ppb oily, sweet 100 ppb green, pond-like, metallic 50 ppb slightly oily, chemical-like

Table II continued

Compound	Detection Threshold	Odor and Flavor Characteristics
<u>Diisopropyl phenols (continued)</u>		
		1,000 ppb lingering oily, chemical, slight phenolic 2,000 ppb chemical, phenolic, oily
2,5-Diisopropyl phenol	100 ppb in water	Neat: phenolic, medicinal, chemical In water: 100 ppb slightly phenolic, slightly oily
2,6-Diisopropyl phenol	100 ppb in water	Neat: phenolic, medicinal, chemical In water: 100 ppb slightly oily
3,5-Diisopropyl phenol	-	Neat: phenolic; cleaning compound-like in low concentrations, iodine-line
<u>Methyl isopropyl phenols</u>		
Thymol (5-methyl-2-(1-methyl ethyl)-phenol)	.014 ppb ²	Neat: phenolic, medicinal; musty, stale at low ppb in air
Carvacrol (2-methyl-5-(1-methyl ethyl)-phenol)	3.6 x 10 ⁻² ppb in air (odor) ² 0.1 ppb in water 100 ppb in fish	Neat: phenolic, medicinal In water: 0.03 ppb stale, phenol 0.3 ppb phenolic 0.1 ppb phenolic, medicinal, sheepy, chemical In fish: 1 ppb sharp, metallic 100 ppb, metallic, peppery, petroleum-like

Table II continued

Compound	Detection Threshold	Odor and Flavor Characteristics
Thiophenol	50 ppb in water (odor) 5 ppb in water (taste) about 15 ppb in fish	Neat: stench, sulfury above 0.25 ppm in air; meaty, brothy, sulfury in low ppb in air In water: 0.1 ppb oily; 10 ppb meaty, rich, brothy In fish: 10 ppb rich, meaty 15 ppb, strange rich, burning, pond- like 20 ppb, burning, old fish oil, stale, papery, rubbery
p-Thiocresol	0.1 ppb (odor) ²	Neat: stench, burnt rubber
p-Methoxythiophenol	-	Neat: stench, burnt plant

¹Data determined in this study unless otherwise referenced.

²Fazzalari, F. A. (38).

³Shumway, D. L; Palensky, J. R. (28).

⁴Rosen, A. A.; et al. (39).

⁵Maga, J. A. (40).

Table III. Frequency of panel responses in flavor identity evaluation of and mean flavor identity scores for tainting flavors in fresh water fish.

Flavor Identity Descriptors	Assigned Value	Naturally Tainted ^a	Spiked Samples	
			1	2
(- Frequency of Responses -)				
Definitely the same type flavor as "R," ^b but much stronger in intensity.	7	3	6	1
Definitely the same type flavor as "R," and is nearly the same intensity.	6	8	6	7
Definitely the same type flavor as "R," but is moderately less intense than "R."	5	2	3	6
Probably the same type flavor as "R," but differs in either intensity and/or flavor quality.	4	5	3	5
Undecided; not sure one way or the other.	3	1	1	2
Probably not the same type flavor as "R"; can only perceive some vague similarities.	2	7	5	8
Definitely not the same type flavor as "R."	1	4	6	1
Flavor Identity Mean Score ^d .		4.00 ^c	4.13 ^c	4.07 ^c

^a Naturally tainted Mosinee Flowage northern pike.

^b "R": Reference: Mosinee northern pike, capture date: June 22, 1979.

^c Mean scores with the same superscript are not different from each other at the 5% level.

^d n = 30.

¹ Rainbow Flowage northern pike plus: 1 ppb 3-isopropyl phenol; 1 ppb 2,4-diisopropyl phenol; 1 ppb carvacrol; 10 ppb thiophenol.

² Rainbow Flowage northern pike plus: 2.5 ppb 3-isopropyl phenol; 2.5 ppb 2,4-diisopropyl phenol; 2.5 ppb carvacrol; 10 ppb thiophenol.

Table IV. Frequency of panelist responses in flavor identity evaluation of and mean flavor identity scores for tainting flavors in rainbow trout.

Flavor Identity Descriptors	Exposure-Tainted Rainbow Trout Samples			
	Tank 1 ^a	Tank 2 ^b	Tank 3 ^c	Tank 4 ^d
	(- - - - Frequency of responses ^e - - - -)			
Definitely the same type flavor as "R," ^f but much stronger in intensity.	3	0	9	5
Definitely the same type flavor as "R," and is nearly the same intensity.	9	9	3	4
Definitely the same type flavor as "R," but is moderately less intense than "R."	3	7	1	3
Probably the same type flavor as "R," but differs in either intensity and/or flavor quality.	4	6	2	6
Undecided; not sure one way or the other.	4	1	0	0
Probably not the same type flavor as "R"; can only perceive some vague similarities.	3	3	5	5
Definitely not the same type flavor as "R."	4	4	10	7
Flavor Identity Mean Score	4.27 ^g	4.20 ^g	3.80 ^g	3.83 ^g

^aOne part Tank 1 exposed to: 100 ppb 3-isopropyl phenol plus 3 parts control trout.

^bOne part Tank 2 exposed to: 33.3 ppb thiophenol plus 3 parts control trout.

^cOne part Tank 3 exposed to: 33.3 ppb thiophenol, 100 ppb 3-isopropyl phenol plus 3 parts control trout.

^dOne part Tank 4 exposed to: 33.3 ppb thiophenol, 100 ppb 3-isopropyl phenol, 250 ppb carvacrol, 250 ppb 2,4-diisopropyl phenol, plus 3 parts control trout.

^e_n = 30.

^f"R": Reference: Mosinee Flowage northern pike, capture date: June 7, 1979.

^gMean scores with the same superscript are not different from each other at the 5% level.

Table V. Descriptive sensory evaluation of yellow perch exposed to foul condensate from a paper mill.

Samples	Sample attribute	
	Off-flavor intensity ¹	Overall preference ²
	(- - - - - Mean scores ³ - - - - -)	
Control, unexposed	2.15 ^a	4.76 ^a
0.1% foul condensate for 24 hours	2.54 ^{a,b}	4.67 ^a
0.2% foul condensate for 24 hours	2.76 ^b	4.49 ^a

¹Scale: 1 = none; 7 = extremely pronounced.

²Scale: 1 = dislike extremely; 7 = like extremely.

³n = 26.

^{a,b}Mean scores in the same column with the same superscript are not statistically different at the 5% level.

Table VI. Descriptive sensory analysis of walleye pike obtained from study sections of the Upper Wisconsin River in the Spring and Fall, 1980.

Sample description	Sample attribute	
	Off-flavor ¹	Preference ²
	(- - - - - Mean Scores ³ - - - - -)	
Spring		
Headwaters #2	2.39 ^a	4.77 ^a
Downstream #3	3.78 ^b	3.63 ^b
Downstream #4	4.46 ^c	3.05 ^c
Downstream #5	4.02 ^{b,c}	3.35 ^{b,c}
Fall		
Headwaters #2	3.69 ^a	3.29 ^a
Downstream #3	3.33 ^a	3.39 ^a
Downstream #4	3.42 ^a	3.49 ^a
Downstream #5	3.35 ^a	3.62 ^a

¹ Scale: 1 = none; 7 = extremely pronounced.

² Scale: 1 = dislike very much; 7 = like very much.

³ n = (Spring) 29
(Fall) 57

a, b, c. Mean scores in the same column with the same superscript are not significant at the 5% level. Comparisons are valid only between samples from the same test session.

Table VII. Selected suggested mechanisms for formation of tainting compounds from diterpene precursors (I, II) shown in Figure 4.

Compound	Precursor and Skeletal Reactions
Cresols (methyl phenols)	I; 8-9 ring cleavage; 9-15 ring closure; aromatization of 9 through 15 ring.
Xylenols (dimethyl phenols)	I; 11-12 and 5-10 ring cleavage; 5-11 ring closure; aromatization of 5 through 11 ring.
Xylenols (dimethyl phenols)	I; 5-10 ring cleavage; 19-10 ring closure; aromatization of 1,2,3,4,19,10 ring.
Methyl isopropyl phenol (carvacrol)	II; 6-7 ring cleavage; 3-6 ring closure; aromatization of 1,2,3,6,5,10 ring.
Isopropyl phenols	II; 7-8 cleavage; aromatization of 8 through 16 ring structure.
Diisopropyl phenols	II; 4/19/20 alkyl migration to position 11; 7-8 cleavage; aromatization of 8 through 16 ring.
2-Methyl isoborneol	I or II; 7-8 cleavage; 3-6 and 4-10 ring closure.
Geosmin	I; 5-10 cleavage; 5-15 and 8-14 ring closures.
Cadin-4-ene-1-ol	I; 11-12 cleavage; 3-6 and 5-11 ring closure.

Figure 1.

Seasonal and capture-site effects on relative concentrations of 3- and 4-isopropyl phenols, 3,4-dimethyl phenol and carvacrol (I_E region - 15.25) in carbowax 20 M GC analysis of walleye pike flavor extracts obtained with equivalent injection sizes and GC sensitivities (A. Rainbow Flowage, capture date: June 22, 1979; B. Lake Wausau, capture date: June 1, 1979; C. Mosinee Flowage, capture date: June 22, 1979; D. Mosinee Flowage, capture date: July 11, 1979).

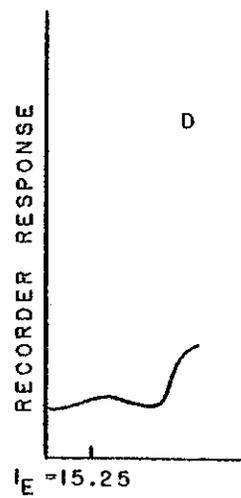
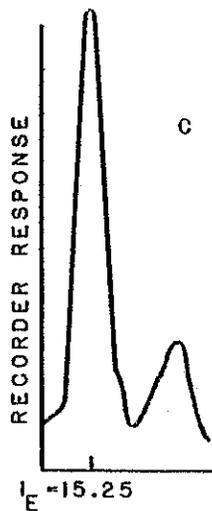
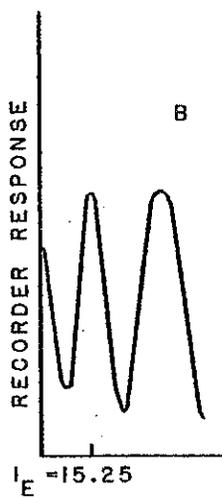
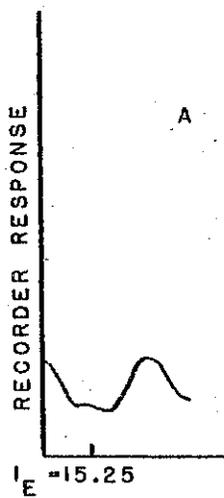


Figure 2.

Generalized biosynthetic pathway for immediate mono-, sesqui-, and diterpene precursors.

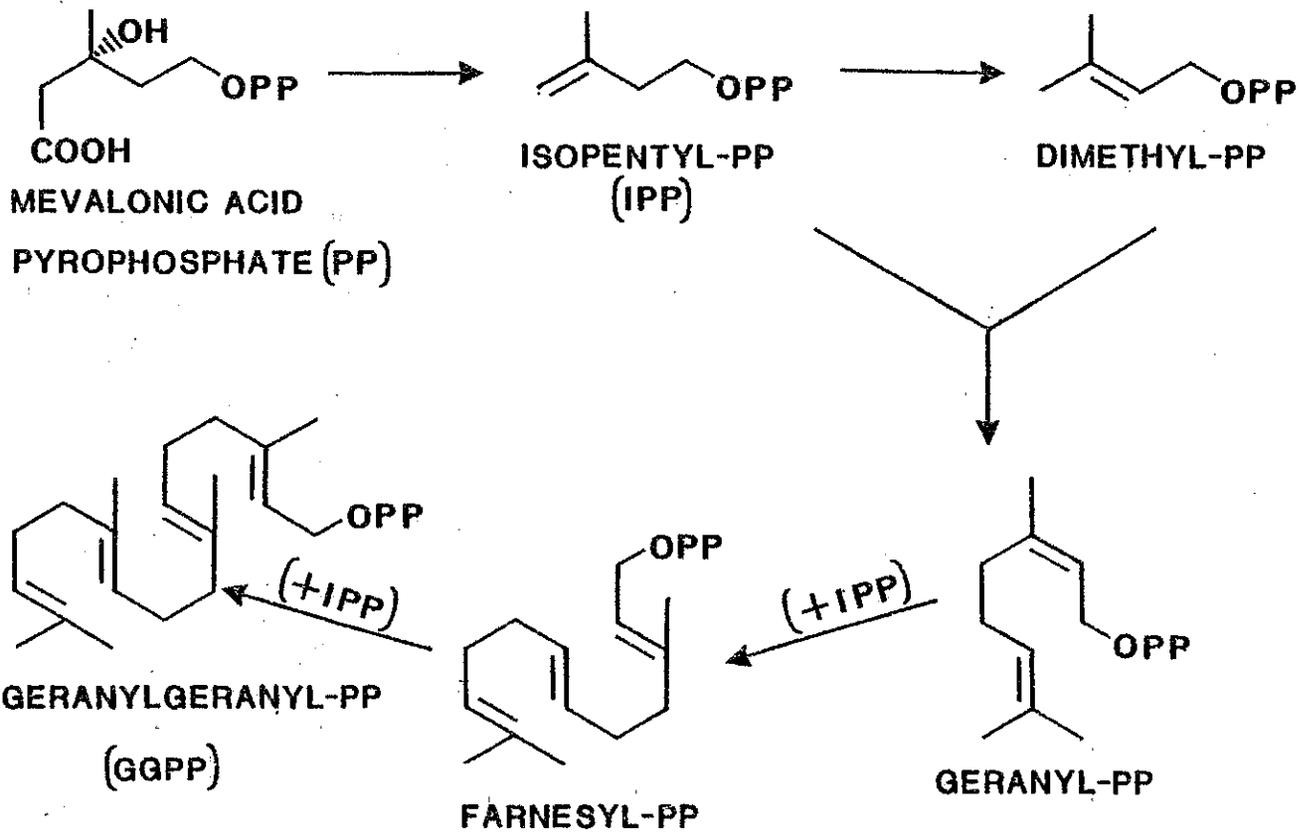


Figure 3

Structural relationships between resin acids and other diterpene compounds.

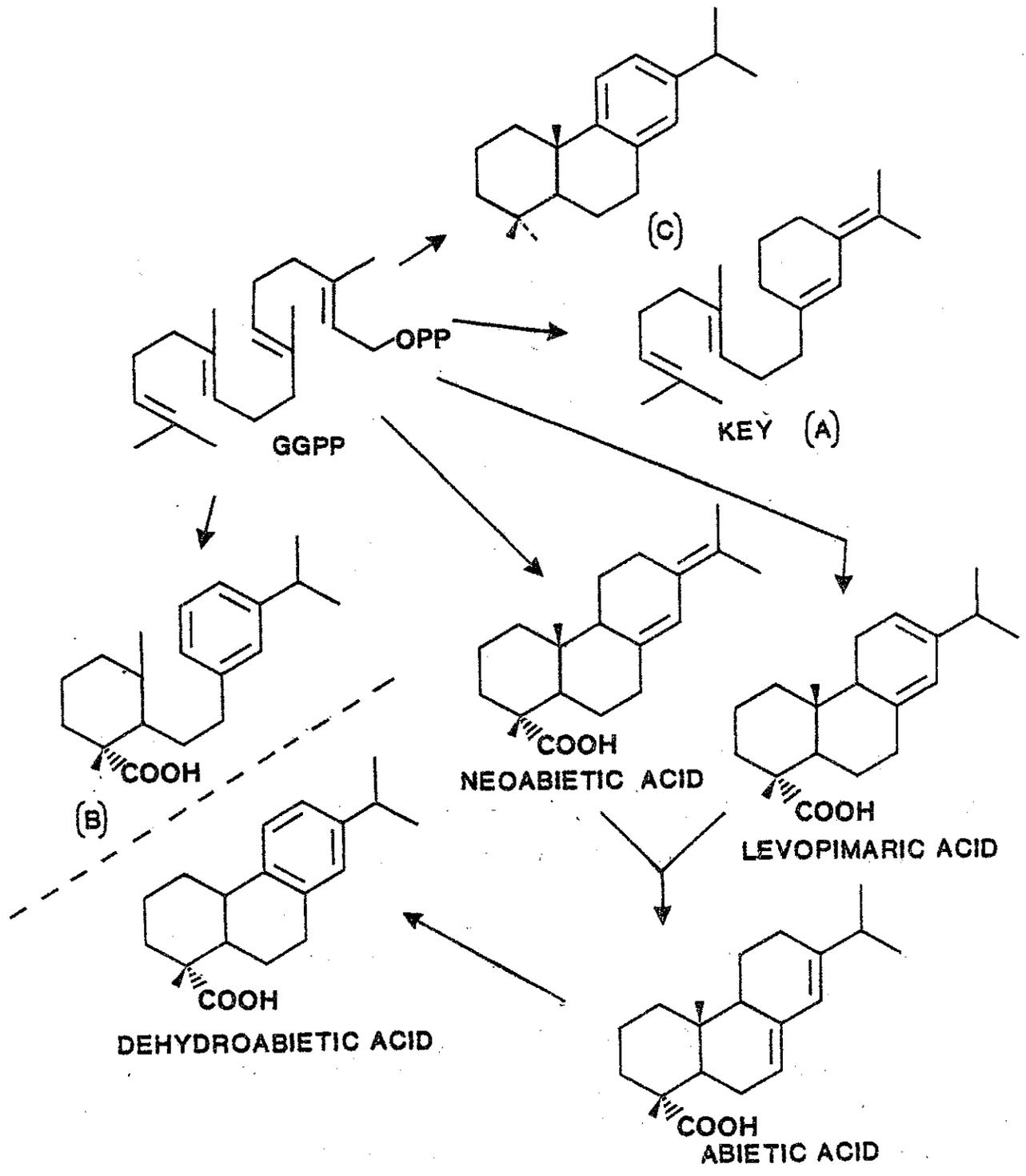


Figure 4.

Postulated unified diterpene system for alkyl phenols and mud-like aroma compounds found in tainted walleye pike.

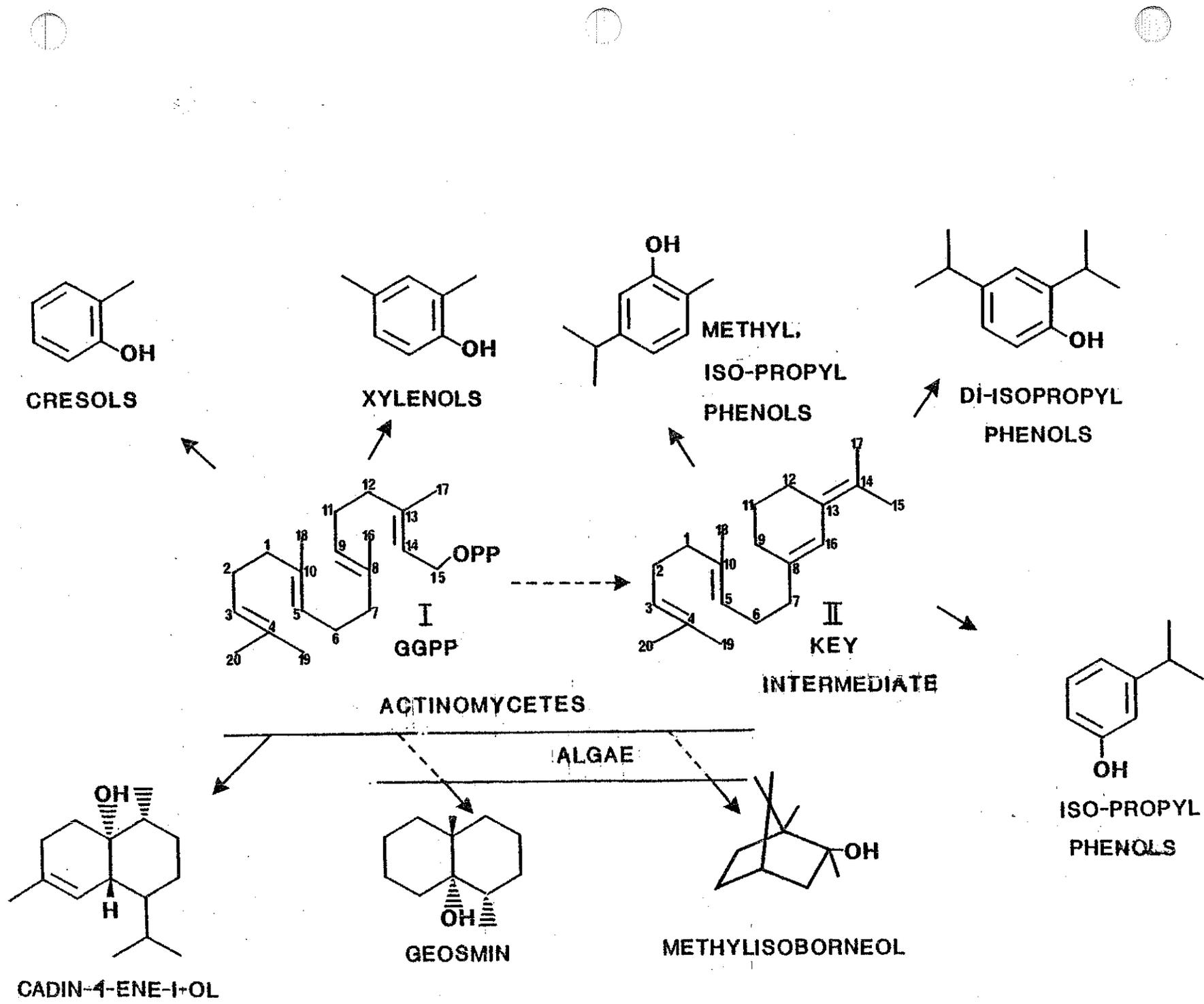


Figure 5.

Schematic showing potential generalized derivations of phenolics compounds from lignin-like structures.

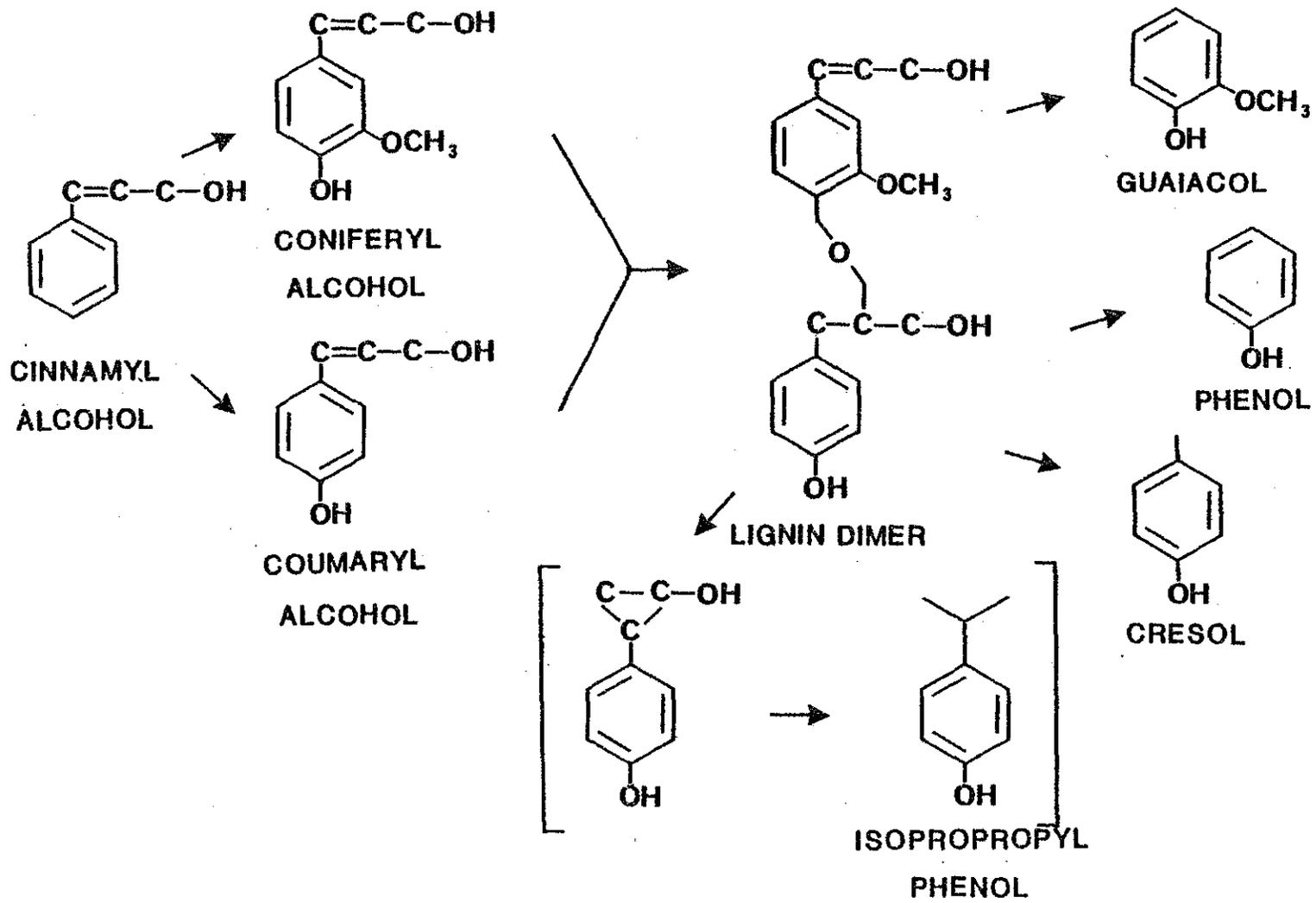
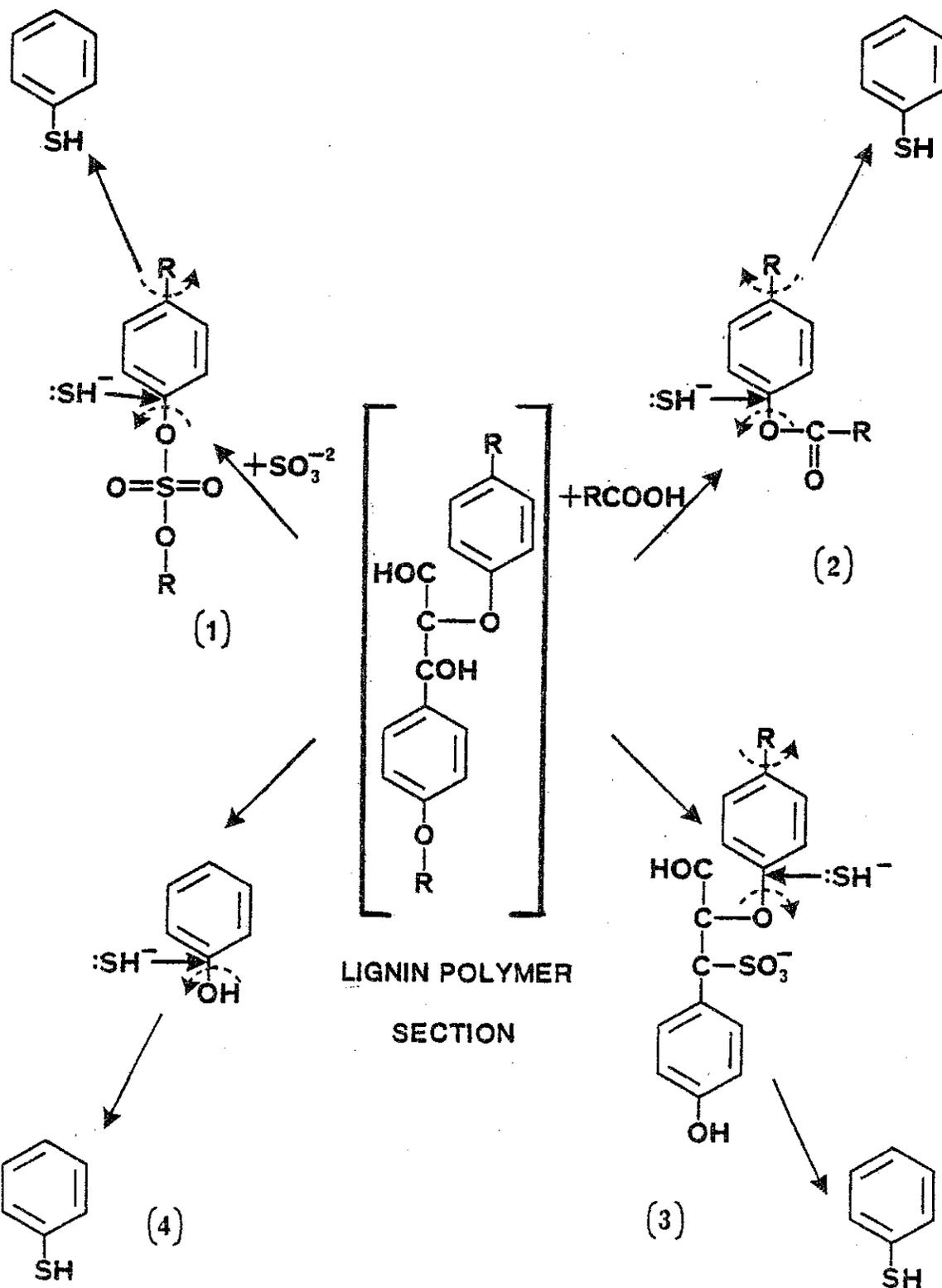


Figure 6.

Suggested routes for the formation of thiophenol from lignin
polymers during alkaline pulping of wood.



Chapter 4

CONCLUSIONS

CONCLUSIONS

During early summer, 1979, walleye pike, northern pike, black crappie, yellow perch and bullheads were captured from three sites on the Upper Wisconsin River; Rainbow Flowage, the upstream site; Mosinee Flowage and Lake Wausau, the downstream sites. Descriptive sensory analysis showed that all sportsfish in the downstream study site were flavor-impaired.

Aromas noted in the gas chromatographic analysis of walleye pike flavor extracts revealed particularly offending areas that had phenolic, medicinal, or chemical aromas; objectionable sulfury, burnt plant, rubber aromas; and musty, earthy, beet-like aroma areas. These same sets of descriptors were used by panel members to describe flavors they observed in the fish flesh.

Gas chromatography-mass spectrometry analysis was used to identify compounds contributing to the overall flavor of walleye pike. Many alkyl phenols, including cresols, dimethyl phenols (xylenols), isopropyl phenols and methyl-isopropyl phenols were identified in regions corresponding to those having phenolic, medicinal or chemical aromas associated with them. While many of these compounds can be linked to plant sources, they also appeared to be contributed by effluents from pulp and paper mills, the only industry along the Upper Wisconsin River. Sulfury, rubbery, burnt plant aromas were found to be caused by aromatic thiol compounds in the flavor extracts, and also appear to be of industrial origin. The musty, muddy, earthy, beet-like aromas noted in the gas chromatograph effluent were found

to be caused by geosmin and 2-methylisoborneol which are known metabolic products of blue-green algae.

Evaluations of flavor characteristics of phenols and aromatic thiols resulted in the selection of combinations of 3-isopropyl phenol, 2,4-diisopropyl phenol, carvacrol and thiophenol which, when added back to broiled fish flesh in appropriate concentrations, gave a flavor impression similar to that observed in off-flavored fish samples from downstream sites.

Accumulation of implicated compounds was demonstrated by static exposure of live trout to selected compositions of tainting mixtures for 24 hrs. Sensory analysis showed that, overall, panelists perceived trout exposed to the selected combinations of tainting compounds to exhibit flavor characteristics similar to naturally tainted fish from the downstream Mosinee Flowage site. Attempts to taint perch with foul condensate from a paper mill resulted in modest off-flavors which were not similar to tainting observed in fish from downstream study sites.

Sensory evaluations of walleye pike captured in late Spring and early Fall revealed a strong seasonal effect on off-flavor intensity. Strong phenolic, medicinal, and chemical flavors were observed in Spring and were related to a build-up of alkyl phenol compounds in the fish flesh. Earthy, musty, muddy flavors observed in both headwaters and downstream samples in Fall, were caused by a dominance of microbial metabolites, geosmin and methylisoborneol.

A diterpene precursor system was proposed which could explain the presence of both alkyl phenols and microbial metabolites.

Diversion of the diterpene precursor model to appropriate end products according to season of the year can be used to explain dominance of phenol flavors in Spring and early Summer, and the dominance of earthy, muddy, microbial metabolite flavors in late Summer and early Fall.

The formation of aromatic thiols was proposed through reactions involving lignin during pulp processing. Detection thresholds for thiophenol was found to be low in fish, thus minor side reactions could be responsible for the formation of these compounds and their occurrence in the fish flesh.

Alkyl-phenolic off-flavors contributed by pulping operations appear to be controllable if appropriate treatments are applied to effluents. During warm periods of the year, suitable biodegradation and oxidation techniques using activated sludge and aerated lagoons are probably adequate to remove diterpene precursors to levels low enough to prevent tainting problems. During winter, when biological processes slow down, chemical oxidation of alkyl phenols already formed in effluents could be accomplished by hydrogen peroxide or potassium permanganate. Any aromatic thiols formed could be removed also from waste streams by oxidation with hydrogen peroxide. During cold periods it might be necessary to enhance procedures used to remove diterpene precursors to prevent formation of alkyl phenols in the river at a later time.

Further research should be directed toward the development of rapid analytical techniques which will enable the detection and quantitation of the alkyl phenols and aromatic thiols implicated

as primary causative agents in fish-tainting. Additional studies should also be directed at confirming the roles and proposed mechanisms of formation of tainting-compounds and these should be related to overall effects of environmental accumulations of these compounds.

1991 fish flavor tainting study progress report to WDNR

→ T. Sheffy WQM/2

SECOND INTERIM PROGRESS REPORT
ON THE
IDENTIFICATION OF OFF-FLAVOR COMPOUND SOURCES FOR FISH AND WATER
FROM THE UPPER WISCONSIN RIVER (BROKAW TO DUBAY DAM)

FROM THE
DEPARTMENT OF FOOD SCIENCE
COLLEGE OF AGRICULTURAL AND LIFE SCIENCES
UNIVERSITY OF WISCONSIN-MADISON
MADISON, WI 53706

PRINCIPAL INVESTIGATOR:
ROBERT C. LINDSAY
PROFESSOR OF FOOD SCIENCE

SUBMITTED TO:
MR. BOB MARTINI
TASK FORCE LEADER
UPPER WISCONSIN RIVER 208 PLAN
DEPARTMENT OF NATURAL RESOURCES
(STATE OF WISCONSIN) BOX 818
RHINELANDER, WI 54501

Date: March 30, 1981

SUMMARY OF PROGRESS

The methods of analysis for volatile flavor compounds in walleye pike were described in the earlier Interim Progress Report dated February 27, 1980 along with detailed information on the sensory analysis of fish for flavor impairment. Work in the ensuing period has focused on the identification of volatile compounds recovered from Rainbow Lake, Wausau, and Mosinee walleye pike, and this work is continuing towards complete summarization in a Master of Science thesis by Nancy Lane. This report describes identifications of compounds based on the best data to date, but due to the nature of the interim status of the report, caution should be maintained in interpretations of compound identities at this point.

Basically, the compounds for which data are available are listed in the accompanying extensive table. Listed in this table are relative retention indices, odors observed at that point in gas chromatographic eluant evaluations, and mass spectral data assessments. Compounds are indicated if the evidence is at least strong enough for tentative identifications.

Many of the heterocyclic compounds are common to cooked flesh and plant foods due to reactions occurring during heating, and contribute to normal and expected flavors of these foods. Similarly, naturally occurring lipid oxidation reactions in the fish prior to and during preparation yield aldehydes, primarily.

Based on a comparison of the data from the control sample location of Rainbow Lake with that from the downstream sites of Wausau and Mosinee, significant tainting compounds have been identified at this time. The gas chromatographic traces provided for fish from the three sites show the relative amounts of compounds indicated, but care must always be exercised in these interpretations because most peaks contain more than

one compound.

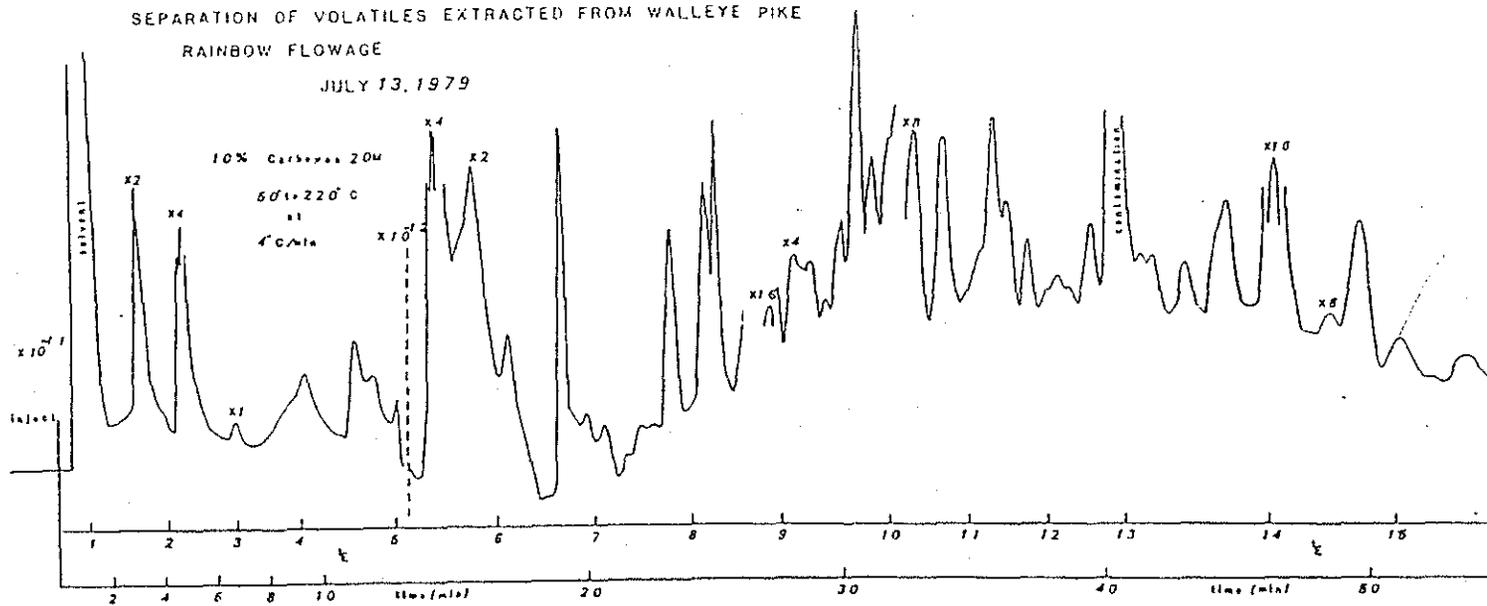
The compounds which have a distinct role in the flavor impairment of Upper Wisconsin River fish are geosmin, methylisoborneol, phenol, cresols, methylisopropyl phenols, isopropyl phenols, and diisopropyl phenols. Most of these compounds have flavor thresholds in very low parts per billion range, and specifically contribute earthy, musty flavors (geosmin, methylisoborneol) or chemical, medicinal flavors (the phenols). Preliminary evaluations of flavor characteristics of compounds in walleye indicate that combinations simulate much of the flavor noted in the naturally flavor-impaired fish.

The mechanisms of formation of compounds shown in the attached series of figures illustrate the common terpene precursor system postulated to account for these interrelated substances. In some instances the formation of the compound involves direct participation of a micro-organism (Streptomycetes, Blue-green algae) while in other situations the reaction could, but not necessarily limited to, proceed on its own. Geranylgeranyl pyrophosphate or a closely-related derivative is likely to be a key in the solution to the tainting problem. This compound is also the direct precursor to abietic acid (rosin acid), and it is likely that plant-derived terpene precursors are fed into the river system from natural vegetation, and possibly industrial sources. We have seen evidence for a seasonal variation in relative proportions of offending compounds, and this is likely due to the ultimate fate of precursors in relation to natural microbial metabolism.

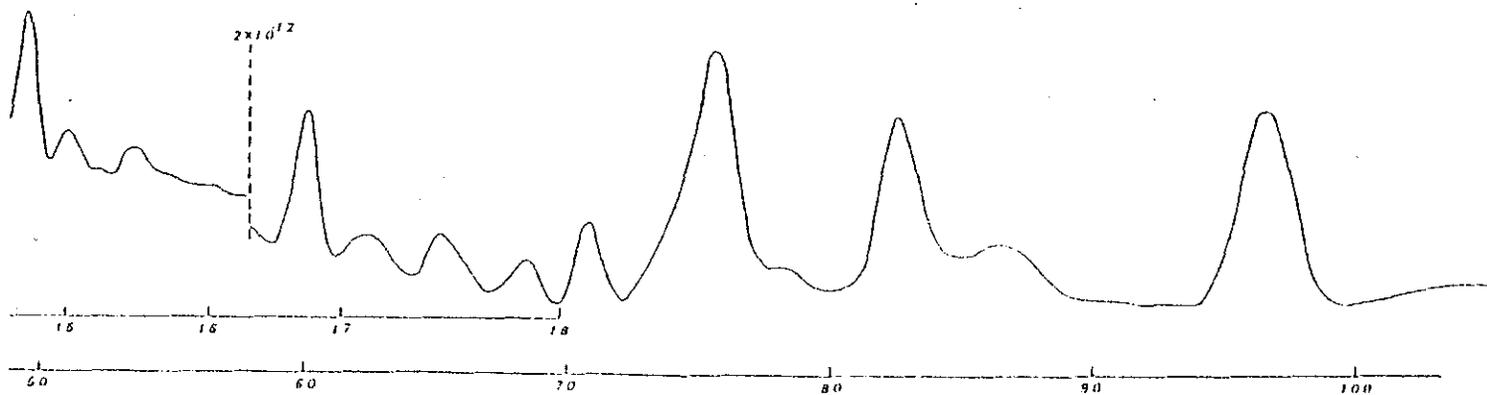
The data are at a stage where there appears to be a clear path of research pursuit for solving the problem of fish-tainting in the river. While the already mentioned compounds definitely contribute to the flavor impairment, other compounds (including terpenes and nitrogen-containing compounds) may ultimately prove equally important in off-flavors.

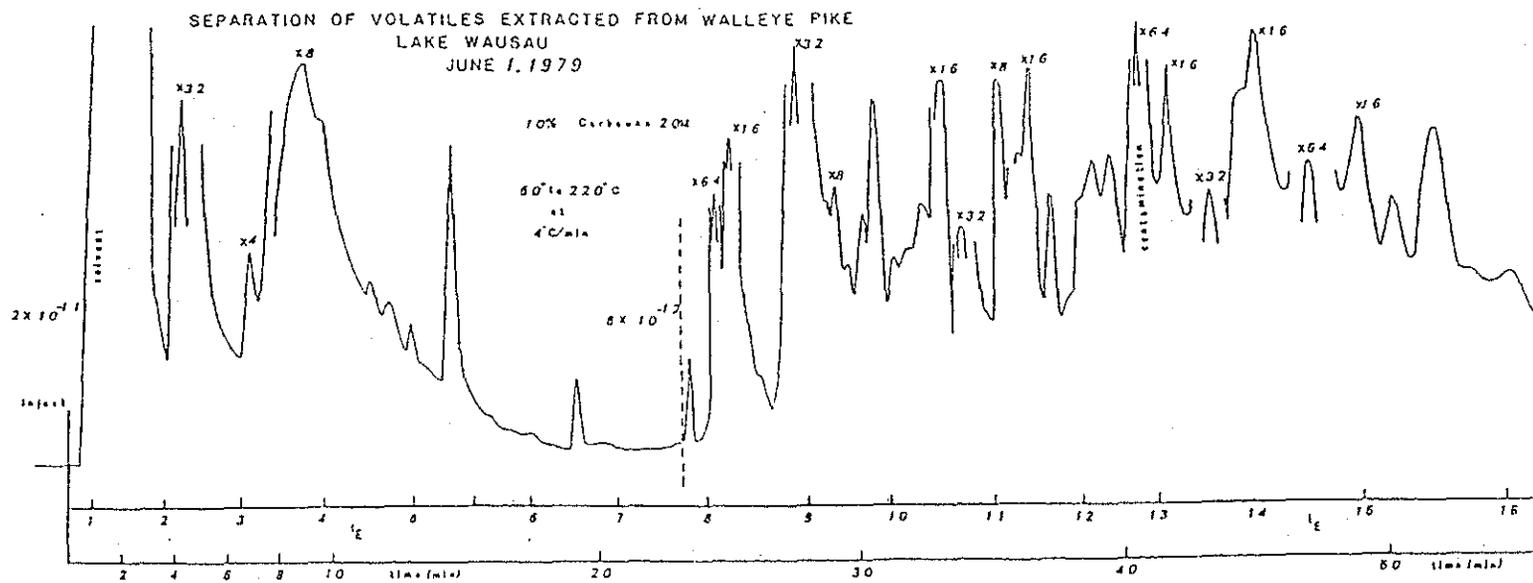
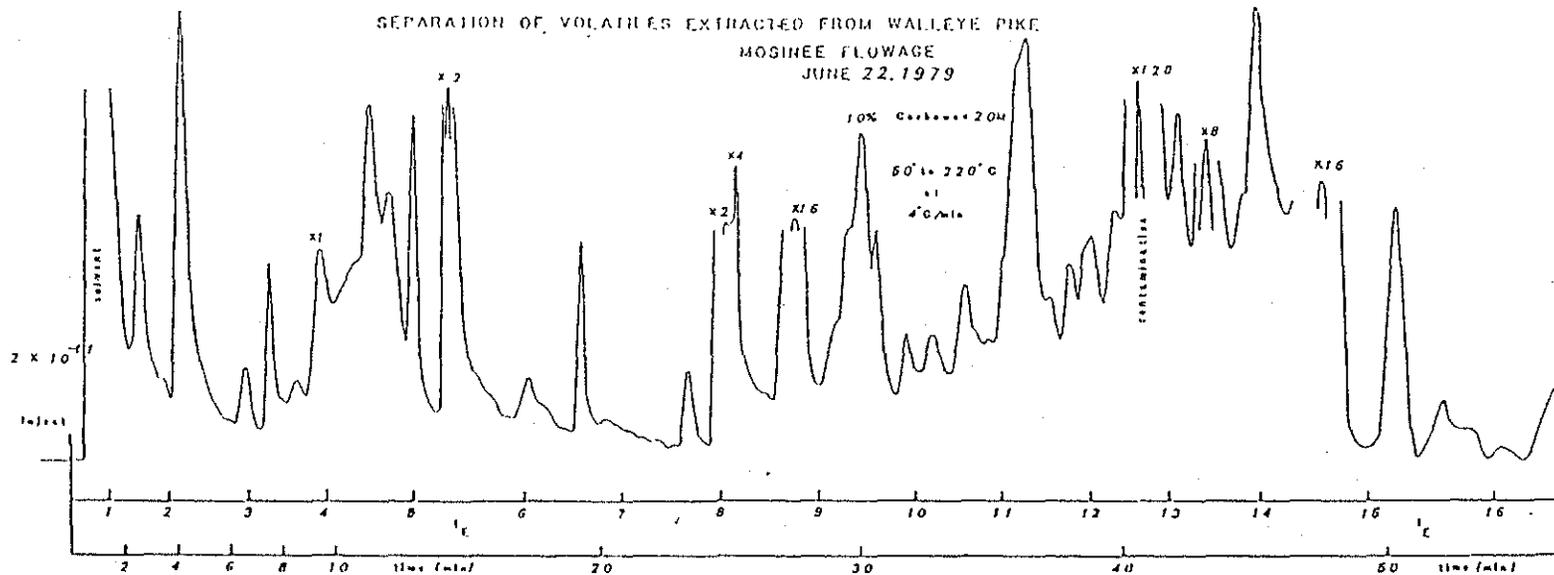
SEPARATION OF VOLATILES EXTRACTED FROM WALLEYE PIKE
RAINBOW FLOWAGE

JULY 13, 1979

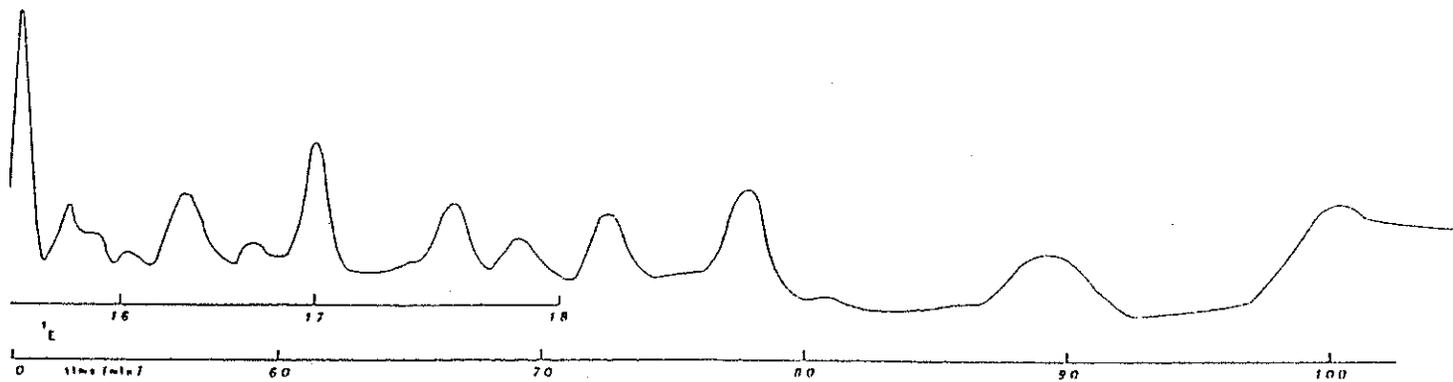


RAINBOW FLOWAGE

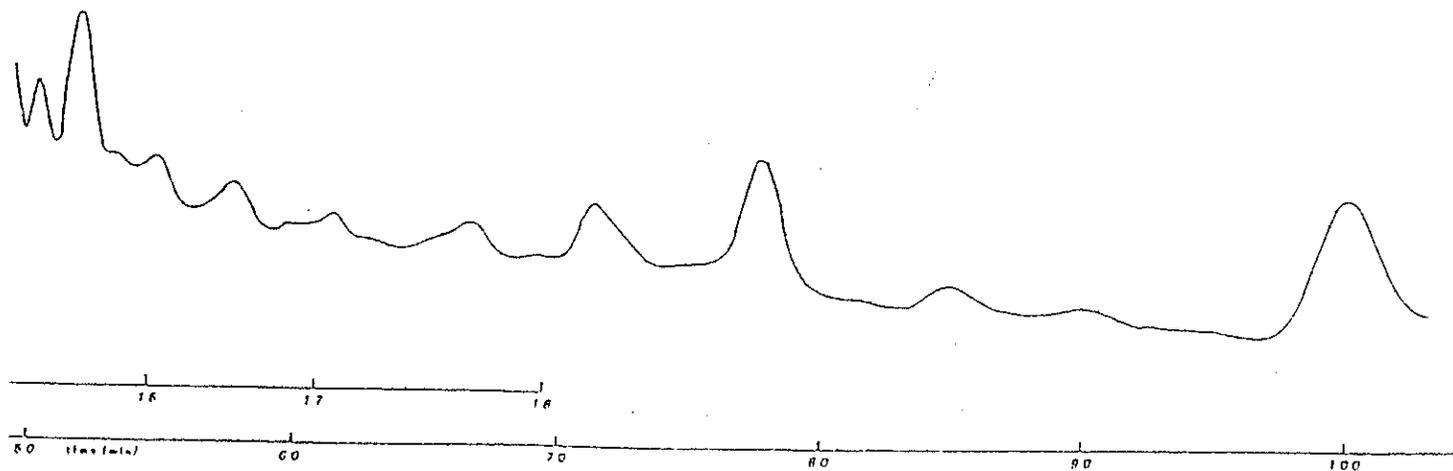




MOSINEE FLOWAGE



LAKE WAUSAU



VOLATILE COMPOUNDS IN FLAVOR EXTRACTS OF WISCONSIN RIVER WALLEYE PIKE

I E	PROBABLE COMPOUND (I _E /M.S.)	SAMPLING SITES								
		RAINBOW FLOWAGE			MOSINEE FLOWAGE			LAKE WAUSAU		
		PRES- ² ENCE	REGION ³ ODOR FROM CHROMATOGRAPH	REL- ⁴ ATIVE PEAK SIZE	PRES- ² ENCE	REGION ³ ODOR FROM CHROMATOGRAPH	REL- ⁴ ATIVE PEAK SIZE	PRES- ² ENCE	REGION ³ ODOR FROM CHROMATOGRAPH	REL- ⁴ ATIVE PEAK SIZE
2.0 to 2.3	Ethyl acetate	+	sweet, estery	VL	+	fruity, estery	VL	+	estery	VL
2.6	Methyl propionate	±	brothy	L	+	brothy	L	±	brothy	L
4.0 to 4.3	alpha-Pinene a methyl silane	-			±	chemical, sharp	M	±	solvent, sharp	L
4.4 to 4.7	Hexanal	+	green, grassy	M	+	grassy	L	+	planty, green	M
4.7 to 5.0	Unknown		skunky	M		skunky	M		skunky	M
5.3	2-methyl-2-Pentenal	+	planty	L	+	spicy, green	L	+	spicy	L

VOLATILE COMPOUNDS IN FLAVOR EXTRACTS OF WISCONSIN RIVER WALLEYE PIKE

I E	PROBABLE COMPOUND (I _E /M.S.)	SAMPLING SITES								
		RAINBOW FLOWAGE			MOSINEE FLOWAGE			LAKE WAUSAU		
		PRES- ² ENCE	REGION ³ ODOR FROM CHROMATOGRAPH	REL- ⁴ ATIVE PEAK SIZE	PRES- ² ENCE	REGION ³ ODOR FROM CHROMATOGRAPH	REL- ⁴ ATIVE PEAK SIZE	PRES- ² ENCE	REGION ³ ODOR FROM CHROMATOGRAPH	REL- ⁴ ATIVE PEAK SIZE
5.6	Heptanal	+	planty	L	+	green, sharp	S	+	sharp	S
5.8	Dodecane	+	chemical	L	+	chemical	S	-		
5.8	Unknown		chemical	L		chemical	S		stale, plastic	S
5.9	beta-Ocimene	+	planty, chemical	VS	+	Planty	M	±	planty	S
6.0 to 6.1	Methyl thiazole	±	green, planty	VS	±	planty	VS	±	planty	VS
6.0 to 6.1	cis-4-Heptenal	±	sharp	VS	±	sharp	M	±	sharp	S

VOLATILE COMPOUNDS IN FLAVOR EXTRACTS OF WISCONSIN RIVER WALLEYE PIKE

		SAMPLING SITES							
		RAINBOW FLOWAGE			MOSINEE FLOWAGE			LAKE WAUSAU	
¹ I E	PROBABLE COMPOUND (I _E /M.S.)	² PRES- ENCE	REGION ³	REL- ⁴	² PRES- ENCE	REGION ³	REL- ⁴	REGION ³	REL- ⁴
			ODOR FROM CHROMATOGRAPH	ATIVE PEAK SIZE		ODOR FROM CHROMATOGRAPH	ATIVE PEAK SIZE	ODOR FROM CHROMATOGRAPH	ATIVE PEAK SIZE
6.4	p-Cymene	-			+	unpleasant, sharp S		-	
6.4 to 6.7	2-Methyl pyrazine	<u>+</u>	popcorn, meaty	VS	-			<u>+</u>	nutty, pyrazine-like VS
6.5 to 6.6	Octanal	+	waxy, soapy	M	+	waxy, soapy	L	+	waxy, soapy M
6.6 to 6.8	Oct-1-en-3-one	<u>+</u>	mushrooms	S	<u>+</u>	mushrooms, musty S		<u>+</u>	musty, mushrooms VS
6.6 to 6.8	t-2-Heptenal	<u>+</u>	aldehyde, sharp	M	<u>+</u>	aldehyde, green	L	<u>+</u>	unsaturated aldehyde M

VOLATILE COMPOUNDS IN FLAVOR EXTRACTS OF WISCONSIN RIVER WALLEYE PIKE

I E	PROBABLE COMPOUND (I _E /M. S.)	SAMPLING SITES							
		RAINBOW FLOWAGE			MOSINEE FLOWAGE			LAKE WAUSAU	
		PRES- ² ENCE	REGION ³ ODOR FROM CHROMATOGRAPH	REL- ⁴ ATIVE PEAK SIZE	PRES- ² ENCE	REGION ³ ODOR FROM CHROMATOGRAPH	REL- ⁴ ATIVE PEAK SIZE	PRES- ² ENCE	REGION ³ ODOR FROM CHROMATOGRAPH
7.29	Ethyl pyrazine 2-Ethylthiazole	-			±	unpleasant, burnt	VS	-	
7.3	4-Ethyl-2-methyl thiazole 2-Methoxythiazole	±	green, planty, unpleasant	VS	±	myrcene-like	VS	-	
7.4	Nonanal	±	aldehyde, soapy	VS	-			-	
7.5 to 7.6	Dimethyl thiazole 2-Ethyl-6-methyl pyrazine	-			±	nutty, sharp, peanutty	VS	-	

VOLATILE COMPOUNDS IN FLAVOR EXTRACTS OF WISCONSIN RIVER WALLEYE PIKE

SAMPLING SITES										
I E	PROBABLE COMPOUND (I _E /M.S.)	RAINBOW FLOWAGE			MOSINEE FLOWAGE			LAKE WAUSAU		
		PRES- ² ENCE	REGION ³ ODOR FROM CHROMATOGRAPH	REL- ⁴ ATIVE PEAK SIZE	PRES- ² ENCE	REGION ³ ODOR FROM CHROMATOGRAPH	REL- ⁴ ATIVE PEAK SIZE	PRES- ² ENCE	REGION ³ ODOR FROM CHROMATOGRAPH	REL- ⁴ ATIVE PEAK SIZE
7.6	2,4-Hexandienal	+	planty	VS	-			-		
7.6 to 7.7	2-Butylthiazole	+	green, oniony	VS	-			-		
7.6 to 7.8	Ethoxythiazole Ethylpyridine	+	unpleasant	VS	+	unpleasant, stale fish tank	VS	+	stale fish tank	VS
7.9	2-Methoxy-3-isopropyl pyrazine	-			+	pyrazine, green	VS	-		

VOLATILE COMPOUNDS IN FLAVOR EXTRACTS OF WISCONSIN RIVER WALLEYE PIKE

		SAMPLING SITES								
		RAINBOW FLOWAGE			MOSINEE FLOWAGE			LAKE WAUSAU		
I ¹ E	PROBABLE COMPOUND (I _E /M.S.)	PRES- ² ENCE	REGION ³	REL- ⁴	PRES- ² ENCE	REGION ³	REL- ⁴	PRES- ² ENCE	REGION ³	REL- ⁴
			ODOR FROM CHROMATOGRAPH	ATIVE PEAK SIZE		ODOR FROM CHROMATOGRAPH	ATIVE PEAK SIZE	ODOR FROM CHROMATOGRAPH	ATIVE PEAK SIZE	
8.0 to 8.1	Isobutylthiazole	+	green tomatoes, green	M	-	-	-	-	-	-
8.0 to 8.1	2,3-Diethylpyrazine	+	musty	VS	+	pyrazine	S	-	-	-
8.04 to 8.2	t-2-Octenal	+	aldehyde	S	-	-	-	-	-	-
8.2	Dimethylpyridine 2-Ethyl-3,5-dimethyl- pyrazine	+	burnt meat	VS	+	pyridine-like	S	+	toasted, pyridine	M
8.4 to 8.5	4-Vinyl pyridine	+	burnt	S	+	pyridine-like	S	+	green	VS

VOLATILE COMPOUNDS IN FLAVOR EXTRACTS OF WISCONSIN RIVER WALLEYE PIKE

SAMPLING SITES										
I E	PROBABLE COMPOUND (I _E /M.S.)	RAINBOW FLOWAGE			MOSINEE FLOWAGE			LAKE WAUSAU		
		PRES- ² ENCE	REGION ³ ODOR FROM CHROMATOGRAPH	REL- ⁴ ATIVE PEAK SIZE	PRES- ² ENCE	REGION ³ ODOR FROM CHROMATOGRAPH	REL- ⁴ ATIVE PEAK SIZE	PRES- ² ENCE	REGION ³ ODOR FROM CHROMATOGRAPH	REL- ⁴ ATIVE PEAK SIZE
8.4	Trimethyl pentene	-			-			+	green, celery	VS
8.43 to 9.09	Methional	+	aldehyde-like	S	+	boiled potatoes	S	+	green, musty	S
8.49	Citronella	-			+	unpleasant, planty, musty	S	+	slight celery musty, green	S
8.6	Indene	-			+	sharp chemical	M	-		
8.7	Benzaldehyde	+	chemical	L	+	sharp chemical	L	-		

VOLATILE COMPOUNDS IN FLAVOR EXTRACTS OF WISCONSIN RIVER WALLEYE PIKE

I E	PROBABLE COMPOUND (I _E /M.S.)	SAMPLING SITES								
		RAINBOW FLOWAGE			MOSINEE FLOWAGE			LAKE WAUSAU		
		PRES- ² ENCE	REGION ³ ODOR FROM CHROMATOGRAPH	REL- ⁴ ATIVE PEAK SIZE	PRES- ² ENCE	REGION ³ ODOR FROM CHROMATOGRAPH	REL- ⁴ ATIVE PEAK SIZE	PRES- ² ENCE	REGION ³ ODOR FROM CHROMATOGRAPH	REL- ⁴ ATIVE PEAK SIZE
8.7	Thiophenol	-			+	heavy, sharp chemical, unpleasant	L	+	sharp chemical	S
8.8 to 8.9	Unknown	<u>+</u>	burnt	L	<u>+</u>	heavy, not pleasant	L	<u>+</u>	burnt	L
8.8	Pentadecane	+	burnt	L	+	heavy, not pleasant	L	-		
8.8	2-Isobutyl-3-methoxy- pyrazine	<u>+</u>	bell pepper	S	<u>+</u>	bell pepper	S	<u>+</u>	green, peanut- like	S

VOLATILE COMPOUNDS IN FLAVOR EXTRACTS OF WISCONSIN RIVER WALLEYE PIKE

I E	PROBABLE COMPOUND (I _E /M.S.)	SAMPLING SITES								
		RAINBOW FLOWAGE			MOSINEE FLOWAGE			LAKE WAUSAU		
		PRES- ² ENCE	REGION ³ ODOR FROM CHROMATOGRAPH	REL- ⁴ ATIVE PEAK SIZE	PRES- ² ENCE	REGION ³ ODOR FROM CHROMATOGRAPH	REL- ⁴ ATIVE PEAK SIZE	PRES- ² ENCE	REGION ³ ODOR FROM CHROMATOGRAPH	REL- ⁴ ATIVE PEAK SIZE
9.0	2,5-Diethyl-4-methyl- thiazole	±	green tomatoe leaves	S	-					
9.0 to 9.1	4-Methyl-5-vinylthiazole	±	cooked vegetable	L	±	plant-like	S	±	vegetable S	
9.06	Tetramethylpyrazine	-			±	musty	S	-		
9.1 to 9.3	Nonenal	+	pleasant, aldehyde like	S	+	cucumbers	S	+	floral, green vegetables S	

VOLATILE COMPOUNDS IN FLAVOR EXTRACTS OF WISCONSIN RIVER WALLEYE PIKE

SAMPLING SITES										
1 I E	PROBABLE COMPOUND (I _E /M.S.)	RAINBOW FLOWAGE			MOSINEE FLOWAGE			LAKE WAUSAU		
		PRES- ² ENCE	REGION ³ ODOR FROM CHROMATOGRAPH	REL- ⁴ ATIVE PEAK SIZE	PRES- ² ENCE	REGION ³ ODOR FROM CHROMATOGRAPH	REL- ⁴ ATIVE PEAK SIZE	PRES- ² ENCE	REGION ³ ODOR FROM CHROMATOGRAPH	REL- ⁴ ATIVE PEAK SIZE
9.1 to 9.3	5-Methoxythiazole Diethylmethylpyrazine	±	cooked vegetables	S	±	roasted	S	±	cooked vegetables	S
9.3	5-Methyl furfural	+	cooked vegetables	M	+	roasted, burnt	S	±	cooked carrots	S
9.3 to 9.4	Unknown (m/e 192)	±	green	M	±	roasted, burnt	S	±	cooked carrots, green veg- tables	S
9.3	Diethyl thiophene	±	cooked vegetables	M	+	Cooked vegetables	S	±	cooked, green vegetables	S

VOLATILE COMPOUNDS IN FLAVOR EXTRACTS OF WISCONSIN RIVER WALLEYE PIKE

		SAMPLING SITES								
		RAINBOW FLOWAGE			MOSINEE FLOWAGE			LAKE WAUSAU		
¹ I E	PROBABLE COMPOUND (I _E /M.S.)	² PRES- ENCE	REGION ³ ODOR FROM CHROMATOGRAPH	REL- ⁴ ATIVE PEAK SIZE	² PRES- ENCE	REGION ³ ODOR FROM CHROMATOGRAPH	REL- ⁴ ATIVE PEAK SIZE	² PRES- ENCE	REGION ³ ODOR FROM CHROMATOGRAPH	REL- ⁴ ATIVE PEAK SIZE
9.6 to 9.7	2-Methylisoborneol	±	camphoraceous chemical	L	±	musty, unpleasant	S	±	musty	S
9.6 to 9.8	Unidentified (m/e 110)	-			±	objectionable, sulfury	S	±	objectionable green	S
9.6 to 9.8	5-Methoxy-2-methyl- thiazole	±	planty	S	±	objectionable, sulfury	S	±	objectionable green	S
10.0	2,4-Decadienal	+	burnt chemical	M	+	old fried greasy potatoes	S	+	objectionable	S

VOLATILE COMPOUNDS IN FLAVOR EXTRACTS OF WISCONSIN RIVER WALLEYE PIKE

I E	PROBABLE COMPOUND (I _E /M.S.)	SAMPLING SITES								
		RAINBOW FLOWAGE			MOSINEE FLOWAGE			LAKE WAUSAU		
		PRES- ² ENCE	REGION ³ ODOR FROM CHROMATOGRAPH	REL- ⁴ ATIVE PEAK SIZE	PRES- ² ENCE	REGION ³ ODOR FROM CHROMATOGRAPH	REL- ⁴ ATIVE PEAK SIZE	PRES- ² ENCE	REGION ³ ODOR FROM CHROMATOGRAPH	REL- ⁴ ATIVE PEAK SIZE
10.1 to 10.3	Isovaleric acid	+	very unpleasant, chemical, stale	M	+	old grease, aldehyde	S	+	unpleasant, cheesy	S
10.1 to 10.3	Myrtenol	+	chemical, stale	M	+	stale	S	+	unpleasant	S
10.23	Phenylacetaldehyde	-			+	green, like phenylacetaldehyde	S	-		
10.4	4-Isobutyl-5-methoxy-2- methylthiazole	+	vegetable, pleasant	M	+	planty	S	-		
10.4 to 10.6	Unknown	+	burnt, harsh, meaty	M	+	chemical, unpleas- ant		+	unpleasant, slight floral	S

VOLATILE COMPOUNDS IN FLAVOR EXTRACTS OF WISCONSIN RIVER WALLEYE PIKE

		SAMPLING SITES								
		RAINBOW FLOWAGE			MOSINEE FLOWAGE			LAKE WAUSAU		
I E	PROBABLE COMPOUND (I _E /M.S.)	PRES- ² ENCE	REGION ³	REL- ⁴	PRES- ² ENCE	REGION ³	REL- ⁴	PRES- ² ENCE	REGION ³	REL- ⁴
			ODOR FROM CHROMATOGRAPH	ATIVE PEAK SIZE		ODOR FROM CHROMATOGRAPH	ATIVE PEAK SIZE		ODOR FROM CHROMATOGRAPH	ATIVE PEAK SIZE
10.5 to 10.6	alpha-Caryophyllene	-			+	unpleasant, chemical	S	+	strange chemical	S
10.6	Unknown	<u>+</u>	planty, oniony	M	-			-		
10.6 to 10.9	2-Acetyl-3-methyl pyrazine	-			<u>+</u>	toasted, roasted	L	-		
10.7	Heptadecane	-			+	burnt, musty, chemical, unpleasant	L	+	strange chemical, L unusual, heavy	

VOLATILE COMPOUNDS IN FLAVOR EXTRACTS OF WISCONSIN RIVER WALLEYE PIKE

I E	PROBABLE COMPOUND (I _E /M.S.)	SAMPLING SITES								
		RAINBOW FLOWAGE				MOSINEE FLOWAGE		LAKE WAUSAU		
		PRES- ² ENCE	REGION ³ ODOR FROM CHROMATOGRAPH	REL- ⁴ ATIVE PEAK SIZE	PRES- ² ENCE	REGION ³ ODOR FROM CHROMATOGRAPH	REL- ⁴ ATIVE PEAK SIZE	PRES- ² ENCE	REGION ³ ODOR FROM CHROMATOGRAPH	REL- ⁴ ATIVE PEAK SIZE
10.7 to 10.9	Unknown	±	burnt, harsh chemical	M	±	burnt, musty, floral	L	±	heavy, burnt	VL
10.8 to 10.9	Unknown	-				floral	L		slight floral	L
10.9	p-Ethyl benzaldehyde	-			+	planty, green	L	-		
10.9	m-Ethyl benzaldehyde	±	licorice or anise like	M	-			-		

VOLATILE COMPOUNDS IN FLAVOR EXTRACTS OF WISCONSIN RIVER WALLEYE PIKE

		SAMPLING SITES								
		RAINBOW FLOWAGE			MOSINEE FLOWAGE			LAKE WAUSAU		
¹ I E	PROBABLE COMPOUND (I _E /M.S.)	² PRES- ENCE	³ REGION ODOR FROM CHROMATOGRAPH	⁴ REL- ATIVE PEAK SIZE	² PRES- ENCE	³ REGION ODOR FROM CHROMATOGRAPH	⁴ REL- ATIVE PEAK SIZE	² PRES- ENCE	³ REGION ODOR FROM CHROMATOGRAPH	⁴ REL- ATIVE PEAK SIZE
11.1	1-Octylbenzene	-			±	sheepy, phenol	M	-		
11.1 to 11.2	Unknown	±	burnt vegetable	L	±	burnt, heterocyclic, heavy chemical	M	±	burnt	S
11.23	2,3,5-Trimethyl-5,7- dihydro-5H- cyclopentapyrazine	±	nutty, pyrazine	M	±	toasted, nutty	M	-		
11.35	Unknown								heavy, burnt	M
11.4	Guaiacol	-			±	smokey, slight guaiacol	S	-		

VOLATILE COMPOUNDS IN FLAVOR EXTRACTS OF WISCONSIN RIVER WALLEYE PIKE

I E	PROBABLE COMPOUND (I _E /M.S.)	SAMPLING SITES								
		RAINBOW FLOWAGE				MOSINEE FLOWAGE			LAKE WAUSAU	
		PRES- ² ENCE	REGION ³ ODOR FROM CHROMATOGRAPH	REL- ⁴ ATIVE PEAK SIZE		PRES- ² ENCE	REGION ³ ODOR FROM CHROMATOGRAPH	REL- ⁴ ATIVE PEAK SIZE	PRES- ² ENCE	REGION ³ ODOR FROM CHROMATOGRAPH
11.4	Unknown	±	stewed vegetable	L	±	stewed vegetable	S	-		
11.6	Unknown	±	meaty, vegetables	M	±	green	M	-		
11.66	Unknown	±	burnt, harsh, thiazole-like	M	-			-		
11.6 to 11.7	Unknown	-			±	musty, unpleasant	S	±	musty, unpleasant	S
11.7	Tridecanal	-			±	soapy, aldehyde	S	±	sharp, green	S

VOLATILE COMPOUNDS IN FLAVOR EXTRACTS OF WISCONSIN RIVER WALLEYE PIKE

I _E ¹	PROBABLE COMPOUND (I _E /M.S.)	SAMPLING SITES								
		RAINBOW FLOWAGE			MOSINEE FLOWAGE			LAKE WAUSAU		
		PRES- ² ENCE	REGION ³ ODOR FROM CHROMATOGRAPH	REL- ⁴ ATIVE PEAK SIZE	PRES- ² ENCE	REGION ³ ODOR FROM CHROMATOGRAPH	REL- ⁴ ATIVE PEAK SIZE	PRES- ² ENCE	REGION ³ ODOR FROM CHROMATOGRAPH	REL- ⁴ ATIVE PEAK SIZE
11.7	Unknown	-			-			±	cooling, mint	M
11.85	Hexanoic acid	+	chemical, burnt, meaty, vegetable	S	+	musty, burnt, heavy, unpleasant	S	+	unpleasant, sharp	S
11.8 to 11.9	2,6,6-Trimethyl-1- crotonyl-1,3- cyclohexadiene (Damasconone)	±	roses	S	±	roses	S	±	roses	S
12.0	6,10-Dimethyl-3,5,9- undecatrien-2-one (pseudo-Ionone)	±	floral	S	±	floral	S	±	floral	S
12.0 to 12.3	1,10-Dimethyl-9-decalol (isomers) (Geosmin)	±	earthy, beet-like	S	±	earthy, beet-like	S	±	earthy, beet-like	S

VOLATILE COMPOUNDS IN FLAVOR EXTRACTS OF WISCONSIN RIVER WALLEYE PIKE

		SAMPLING SITES								
		RAINBOW FLOWAGE			MOSINEE FLOWAGE			LAKE WAUSAU		
I E	PROBABLE COMPOUND (I _E /M.S.)	PRES- ² ENCE	REGION ³	REL- ⁴	PRES- ² ENCE	REGION ³	REL- ⁴	PRES- ² ENCE	REGION ³	REL- ⁴
			ODOR FROM CHROMATOGRAPH	ATIVE PEAK SIZE		ODOR FROM CHROMATOGRAPH	ATIVE PEAK SIZE		ODOR FROM CHROMATOGRAPH	ATIVE PEAK SIZE
12.0 to 12.3	Methyl naphthalene	±	earth, beet-like	S	±	earthy, beet-like	S	±	earthy, beet-like	S
12.1 to 12.2	Unknown	±	burnt	S	±	burnt	S	-		
12.1	Unknown (m/e 220)	-				earthy, beet-like	S	-		
12.1	Unknown (m/e 240)	-				earth, beet-like	S	-		
12.2 to 12.3	4-(2,6,6-trimethyl-2- cyclohexen-1-yl)- 3-Buten-2-one (alpha-Ionone)	-			±	semi-floral	S	±	sweet	S

VOLATILE COMPOUNDS IN FLAVOR EXTRACTS OF WISCONSIN RIVER WALLEYE PIKE

		SAMPLING SITES								
		RAINBOW FLOWAGE			MOSINEE FLOWAGE			LAKE WAUSAU		
¹ I E	PROBABLE COMPOUND (I _E /M.S.)	² PRES- ENCE	³ REGION ODOR FROM CHROMATOGRAPH	⁴ REL- ATIVE PEAK SIZE	² PRES- ENCE	³ REGION ODOR FROM CHROMATOGRAPH	⁴ REL- ATIVE PEAK SIZE	² PRES- ENCE	³ REGION ODOR FROM CHROMATOGRAPH	REL- ATIVE PEAK SIZE
12.2	Unknown	-			-			-	musty	S
12.4	Nicotine	-			+	burnt plant	S	-		
12.6	Unknown	-			+	burnt	L	-		
12.63	Unidentified terpene derivative		woody, kitchen matches	L		woody, planty	VL		pine lumber	VL
12.68	Unknown		heavy chemical	L	-			-		

VOLATILE COMPOUNDS IN FLAVOR EXTRACTS OF WISCONSIN RIVER WALLEYE PIKE

		SAMPLING SITES								
		RAINBOW FLOWAGE			MOSINEE FLOWAGE			LAKE WAUSAU		
1 I E	PROBABLE COMPOUND (I _E /M.S.)	PRES- ² ENCE	REGION ³	REL- ⁴	PRES- ² ENCE	REGION ³	REL- ⁴	PRES- ² ENCE	REGION ³	REL- ⁴
			ODOR FROM CHROMATOGRAPH	ATIVE PEAK SIZE		ODOR FROM CHROMATOGRAPH	ATIVE PEAK SIZE		ODOR FROM CHROMATOGRAPH	ATIVE PEAK SIZE
12.8	2,6 di-tert-butyl-4- methyl phenol (Butylated Hydroxy Toluene) (Contaminant)	+	(no odor)	VL	+	(no odor)	VL	+	(no odor)	VL
12.4 to 12.6	Unknown		wet, papery	S	-			-		
12.9	Unknown		planty	M	-			-		
13.0	Unknown		liver, meaty	S	-			-		
13.1	Unknown		stale, heavy	M		chemical	VL		unpleasant	M

VOLATILE COMPOUNDS IN FLAVOR EXTRACTS OF WISCONSIN RIVER WALLEYE PIKE

SAMPLING SITES										
I E	PROBABLE COMPOUND (I _E /M.S.)	RAINBOW FLOWAGE			MOSINEE FLOWAGE			LAKE WAUSAU		
		PRES- ² ENCE	REGION ³ ODOR FROM CHROMATOGRAPH	REL- ⁴ ATIVE PEAK SIZE	PRES- ² ENCE	REGION ³ ODOR FROM CHROMATOGRAPH	REL- ⁴ ATIVE PEAK SIZE	PRES- ² ENCE	REGION ³ ODOR FROM CHROMATOGRAPH	REL- ⁴ ATIVE PEAK SIZE
13.1	1,2,3,4-tetrahydro-1,1,6-trimethyl Naphthalene (beta-Ionene)	±	sweet	M	±	floral, jam	M	±	heavy, sweet	M
13.2 to 13.4	Benzothiazole	+	rubbery, old tennis shoes	S	+	hot rubber, tennis shoes	S	+	rubbery	L
13.2 to 13.4	o-Cresol 2-Methoxy-4-methyl phenol	±	rubbery, old tennis shoes	S	±	hot rubber, tennis shoes	S	±	rubbery	L
13.3	Phenol	-			+	hot rubber, tennis shoes	S	+	rubbery	L
13.3	Unidentified (m/e 140)	-				hot rubber, tennis shoes	S		rubbery	L

VOLATILE COMPOUNDS IN FLAVOR EXTRACTS OF WISCONSIN RIVER WALLEYE PIKE

		SAMPLING SITES								
		RAINBOW FLOWAGE			MOSINEE FLOWAGE			LAKE WAUSAU		
1 I E	PROBABLE COMPOUND (I _E /M.S.)	PRES- ² ENCE	REGION ³	REL- ⁴	REGION ³	REL- ⁴	REGION ³	REL- ⁴	REGION ³	REL- ⁴
			ODOR FROM CHROMATOGRAPH	ATIVE PEAK SIZE	ODOR FROM CHROMATOGRAPH	ATIVE PEAK SIZE	ODOR FROM CHROMATOGRAPH	ATIVE PEAK SIZE	ODOR FROM CHROMATOGRAPH	ATIVE PEAK SIZE
13.6 to 14.0	Epicubenol gamma-Nonalactone	+	ripe berries, sweet, vertaldehyde-like	VL	+	pleasant, coconutty, veratrole-like	M	+	sweet, veratrole-like	M
13.6 to 14.0	Biphenyl	-			+	pleasant, coconutty, veratrole-like	M	+	sweet, veratrole-like	M
13.4	Unknown		burnt, slight floral	S	-			-		
13.4	Unknown	-				stale, bad pond	VL	-		
13.5	Dimethyl Naphthalene	+	no odor	S	+	no odor	S	+	no odor	S

VOLATILE COMPOUNDS IN FLAVOR EXTRACTS OF WISCONSIN RIVER WALLEYE PIKE

SAMPLING SITES										
I ¹ E	PROBABLE COMPOUND (I _E /M.S.)	RAINBOW FLOWAGE			MOSINEE FLOWAGE			LAKE WAUSAU		
		PRES- ² ENCE	REGION ³ ODOR FROM CHROMATOGRAPH	REL- ⁴ ATIVE PEAK SIZE	PRES- ² ENCE	REGION ³ ODOR FROM CHROMATOGRAPH	REL- ⁴ ATIVE PEAK SIZE	PRES- ² ENCE	REGION ³ ODOR FROM CHROMATOGRAPH	REL- ⁴ ATIVE PEAK SIZE
13.7	Unknown		burnt, heavy	M	-					
13.9	Unknown (m/e 180)		sweet, medicinal	L		pleasant, veratrole-like	M		sweet, veratrole-like	M
13.8 to 14.0	t-Cinnamaldehyde	±	spicey, floral	L	-			±	cloves	L
14.0	Unknown (m/e 178)		weak rosiny	L	-		L		soldering rosin	L
14.0 to 14.2	2-Isopropyl phenol Indanone	±	indole-like, medicinal	S	±	indole-like, alkyl phenol	L	±	alkyl phenol	L

VOLATILE COMPOUNDS IN FLAVOR EXTRACTS OF WISCONSIN RIVER WALLEYE PIKE

		SAMPLING SITES								
		RAINBOW FLOWAGE			MOSINEE FLOWAGE			LAKE WAUSAU		
I E	PROBABLE COMPOUND (I _E /M.S.)	PRES- ² ENCE	REGION ³	REL- ⁴	PRES- ² ENCE	REGION ³	REL- ⁴	REGION ³	REL- ⁴	
			ODOR FROM CHROMATOGRAPH	ATIVE PEAK SIZE		ODOR FROM CHROMATOGRAPH	ATIVE PEAK SIZE	ODOR FROM CHROMATOGRAPH	ATIVE PEAK SIZE	
14.1	1,5-di-tert-Butyl-dimethyl- bicyclo (3.1.0) hexanone (odor unknown)				±	alkyl phenol indole-like	L	±	alkyl phenol	L
14.1	Unknown		indole-like, medicinal	S		alkyl phenol indole-like	L		alkyl phenol	L
14.3 to 14.1	Unknown		floral, sweet	M	-			-		
14.5	Unknown		floral, vanilla- like	S	-				chemical	L

VOLATILE COMPOUNDS IN FLAVOR EXTRACTS OF WISCONSIN RIVER WALLEYE PIKE

I _E ¹	PROBABLE COMPOUND (I _E /M.S.)	SAMPLING SITES								
		RAINBOW FLOWAGE			MOSINEE FLOWAGE			LAKE WAUSAU		
		PRES- ² ENCE	REGION ³ ODOR FROM CHROMATOGRAPH	REL- ⁴ ATIVE PEAK SIZE	PRES- ² ENCE	REGION ³ ODOR FROM CHROMATOGRAPH	REL- ⁴ ATIVE PEAK SIZE	PRES- ² ENCE	REGION ³ ODOR FROM CHROMATOGRAPH	REL- ⁴ ATIVE PEAK SIZE
14.5 to 14.9	Unknown (m/e 168)	±	hot, rosiny	VS	±	rosiny, lumber	VL	±	rosin, chemical in	VL
	Unknown (m/e 196)	±		VS	±		VL	±	tail	VL
14.6	Unknown		celery-like, stale	S	-			-		
14.7	Unknown		chemical, burnt	M	±	stale, musty, rubbery air	VL	-		
14.9 to 15.1	Unknown	-				burnt sugar, sweet	M		sweet, burnt jam	M
14.9 to 15.1	Unknown		pleasant, cooked		-			-		

VOLATILE COMPOUNDS IN FLAVOR EXTRACTS OF WISCONSIN RIVER WALLEYE PIKE

I E	PROBABLE COMPOUND (I _E /M.S.)	SAMPLING SITES								
		RAINBOW FLOWAGE			MOSINEE FLOWAGE			LAKE WAUSAU		
		PRES- ² ENCE	REGION ³ ODOR FROM CHROMATOGRAPH	REL- ⁴ ATIVE PEAK SIZE	PRES- ² ENCE	REGION ³ ODOR FROM CHROMATOGRAPH	REL- ⁴ ATIVE PEAK SIZE	PRES- ² ENCE	REGION ³ ODOR FROM CHROMATOGRAPH	REL- ⁴ ATIVE PEAK SIZE
15.3	3-Isoprpyol phenol 1,2-Dimethyl indole 3,4-Dimethyl phenol	+	mild indole- like	M	+	phenolic	L	+	medicinal	L
15.3	2-Methyl-5-(1-methylethyl)- Phenol (Carvacvol)				+	phenolic	L	+	medicinal	L
15.5	Unknown		musty, dusty, stale	M	-			-		
15.5 to 15.9	Unknown Terpene derivative (m/e 194)		spicy	S		woody, piney, rosiny	VVL		piney, rosiny	VVL
15.6	Unknown		heavy chemical	S		heterocyclic	VS		naphthalic, piney	L

VOLATILE COMPOUNDS IN FLAVOR EXTRACTS OF WISCONSIN RIVER WALLEYE PIKE

I E	PROBABLE COMPOUND (I _E /M.S.)	SAMPLING SITES								
		RAINBOW FLOWAGE			MOSINEE FLOWAGE			LAKE WAUSAU		
		PRES- ² ENCE	REGION ³ ODOR FROM CHROMATOGRAPH	REL- ⁴ ATIVE PEAK SIZE	PRES- ² ENCE	REGION ³ ODOR FROM CHROMATOGRAPH	REL- ⁴ ATIVE PEAK SIZE	PRES- ² ENCE	REGION ³ ODOR FROM CHROMATOGRAPH	REL- ⁴ ATIVE PEAK SIZE
15.6	Methyl hexadecanoate	+	little odor	S	+	little odor	VS	+	little odor	L
15.7	Unknown	-			-				musty, camphor	L
15.9	Unknown		celery, cooked vegetable	M	-			-		
15.9	Unknown	-			-				unpleasant chemical	M
16.0	Unknown	-			-				jammy	M

VOLATILE COMPOUNDS IN FLAVOR EXTRACTS OF WISCONSIN RIVER WALLEYE PIKE

SAMPLING SITES										
I ¹ E	PROBABLE COMPOUND (I _E /M.S.)	RAINBOW FLOWAGE			MOSINEE FLOWAGE			LAKE WAUSAU		
		PRES- ² ENCE	REGION ³ ODOR FROM CHROMATOGRAPH	REL- ⁴ ATIVE PEAK SIZE	PRES- ² ENCE	REGION ³ ODOR FROM CHROMATOGRAPH	REL- ⁴ ATIVE PEAK SIZE	PRES- ² ENCE	REGION ³ ODOR FROM CHROMATOGRAPH	REL- ⁴ ATIVE PEAK SIZE
16.0	Unknown (m/e 250)		musty	S		stale, chemical	S		jammy, unpleasant	M
16.0	Ethyl hexadecanoate	+	little odor	S	+	little odor	S	+	little odor	M
16.0 16.1	Unknown (m/e 256)		musty	S		stale, chemical	S		jammy, unpleasant	M
16.1 to 16.4	Cinnamyl alcohol	±	pleasant, peppery, wet, cinnamon	S	±	spicy, peppery	L	±	peppery	M
16.2	Unknown	-				chemical	S	-		

VOLATILE COMPOUNDS IN FLAVOR EXTRACTS OF WISCONSIN RIVER WALLEYE PIKE

		SAMPLING SITES								
		RAINBOW FLOWAGE			MOSINEE FLOWAGE			LAKE WAUSAU		
¹ I E	PROBABLE COMPOUND (I _E /M.S.)	² PRES- ENCE	³ REGION ODOR FROM CHROMATOGRAPH	⁴ REL- ATIVE PEAK SIZE	² PRES- ENCE	³ REGION ODOR FROM CHROMATOGRAPH	⁴ REL- ATIVE PEAK SIZE	² PRES- ENCE	³ REGION ODOR FROM CHROMATOGRAPH	⁴ REL- ATIVE PEAK SIZE
16.4	Unknown (m/e 276)		plant-like	S		black pepper	L		fatty, stale, musty	M
16.4 to 16.6	Unknown		clear musk	S		pleasant, sweet	S		sweet	M
16.5	Unknown (m/e 258)	-				black pepper	L		muttoney, fatty, musty, stale	M
16.5	Tricosane	+	no odor	S	+	no odor	S	+	no odor	M
16.5	Unknown	-				cleaning compound	S	-		
16.5 to 16.6	Unknown	-				iodine-like	M		iodine-like musty	M

VOLATILE COMPOUNDS IN FLAVOR EXTRACTS OF WISCONSIN RIVER WALLEYE PIKE

		SAMPLING SITES							
		RAINBOW FLOWAGE			MOSINEE FLOWAGE			LAKE WAUSAU	
I E	PROBABLE COMPOUND (I _E /M.S.)	PRES- ² ENCE	REGION ³	REL- ⁴	PRES- ² ENCE	REGION ³	REL- ⁴	REGION ³	REL- ⁴
			ODOR FROM CHROMATOGRAPH	ATIVE PEAK SIZE		ODOR FROM CHROMATOGRAPH	ATIVE PEAK SIZE	ODOR FROM CHROMATOGRAPH	ATIVE PEAK SIZE
16.6	Unknown		chemical	VS	-				
16.6	Unknown	-				piney	S	-	
16.6	Diethyl phthalate	+	diethyl phthalate like	L	+		S	+	M
16.7	Unknown		plant-like	S	-			-	
16.7	Unknown		burnt hair	VS	-			-	
16.9	Unknown	-				heavy chemical	M	-	

VOLATILE COMPOUNDS IN FLAVOR EXTRACTS OF WISCONSIN RIVER WALLEYE PIKE

I E	PROBABLE COMPOUND (I _E /M.S.)	SAMPLING SITES								
		RAINBOW FLOWAGE			MOSINEE FLOWAGE			LAKE WAUSAU		
		PRES- ² ENCE	REGION ³ ODOR FROM CHROMATOGRAPH	REL- ⁴ ATIVE PEAK SIZE	PRES- ² ENCE	REGION ³ ODOR FROM CHROMATOGRAPH	REL- ⁴ ATIVE PEAK SIZE	PRES- ² ENCE	REGION ³ ODOR FROM CHROMATOGRAPH	REL- ⁴ ATIVE PEAK SIZE
17.0	Ethyl heptadecanoate	+	little odor	S	+	little odor	L	+	little odor	S
17.1	Unknown	-				planty, hot, sharp	M	-		
17.1 to 17.41	Unknown		sweet	S		sweet, pleasant	M		burnt vanillin	M
17.5	Methyl octadecanoate	+	little odor	M	+	little odor	M	+	little odor	M
17.6	Unknown	-				hot	L		sweet	M
18.1	Unknown	-				very soapy	M	-		

VOLATILE COMPOUNDS IN FLAVOR EXTRACTS OF WISCONSIN RIVER WALLEYE PIKE

SAMPLING SITES										
I E	PROBABLE COMPOUND (I _E /M.S.)	RAINBOW FLOWAGE			MOSINEE FLOWAGE		LAKE WAUSAU			
		PRES- ² ENCE	REGION ³ ODOR FROM CHROMATOGRAPH	REL- ⁴ ATIVE PEAK SIZE	PRES- ² ENCE	REGION ³ ODOR FROM CHROMATOGRAPH	REL- ⁴ ATIVE PEAK SIZE	PRES- ² ENCE	REGION ³ ODOR FROM CHROMATOGRAPH	REL- ⁴ ATIVE PEAK SIZE
18.2	Unknown	-				cooked vegetables	M		pleasant, plant- like	M
18.3	Unknown	-			-				rosiney, woody, piney	L
18.35	Vanillin	-			+	vanilla-like	M	+	vanilla-like	M
>18	Diisobutyl phthalate	-			+			+		
>>18	Diethyl phthalate	-			+			+		

VOLATILE COMPOUNDS IN FLAVOR EXTRACTS OF WISCONSIN RIVER WALLEYE PIKE

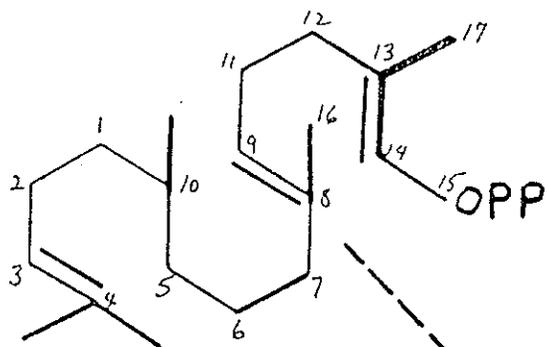
		SAMPLING SITES								
		RAINBOW FLOWAGE			MOSINEE FLOWAGE			LAKE WAUSAU		
¹ I E	PROBABLE COMPOUND (I _E /M.S.)	² PRES- ENCE	REGION ³	REL- ⁴	² PRES- ENCE	REGION ³	REL- ⁴	² PRES- ENCE	REGION ³	REL- ⁴
			ODOR FROM CHROMATOGRAPH	ACTIVE PEAK SIZE		ODOR FROM CHROMATOGRAPH	ACTIVE PEAK SIZE		ODOR FROM CHROMATOGRAPH	ACTIVE PEAK SIZE

¹I_E: Retention indices relative to standard ethyl esters on Carbowax 20 M columns.

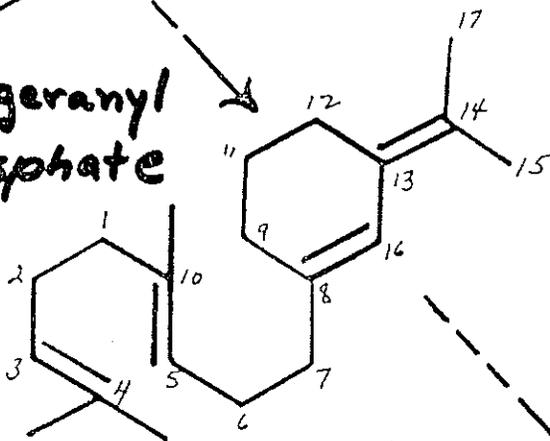
²Presence: + Presence indicated by mass spectra and gas chromatographic retention indices correlation.
 + Presence indicated by mass spectra or gas chromatographic retention indices and odor associated with the peak as it eluted from the Carbowax column.
 - Presence not indicated by mass spectra or gas chromatographic retention indices and odor associated with the peak as it eluted from the Carbowax column. Note that the compound may still be present, its characteristic odor may be masked by others coeluting.

³Region Odor From Chromatograph: The detector system of the gas chromatograph was modified with an effluent splitter to allow simultaneous use of flame ionization detector, and evaluation of peak effluents during chromatographic analysis.

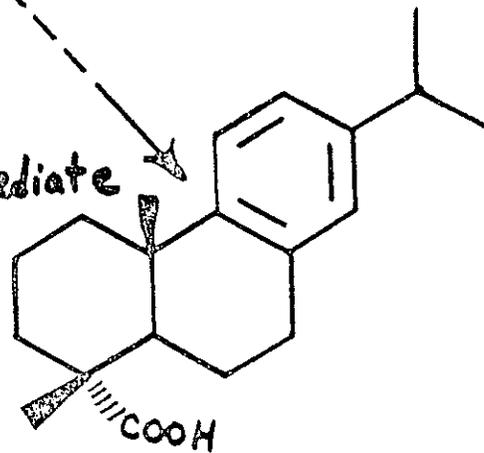
⁴Relative peak size: The size of a specific peak compared to the total area of the gas chromatographic pattern for the specified sample.
 VS very small
 S small
 M medium
 L large
 VL very large
 VVL very, very large



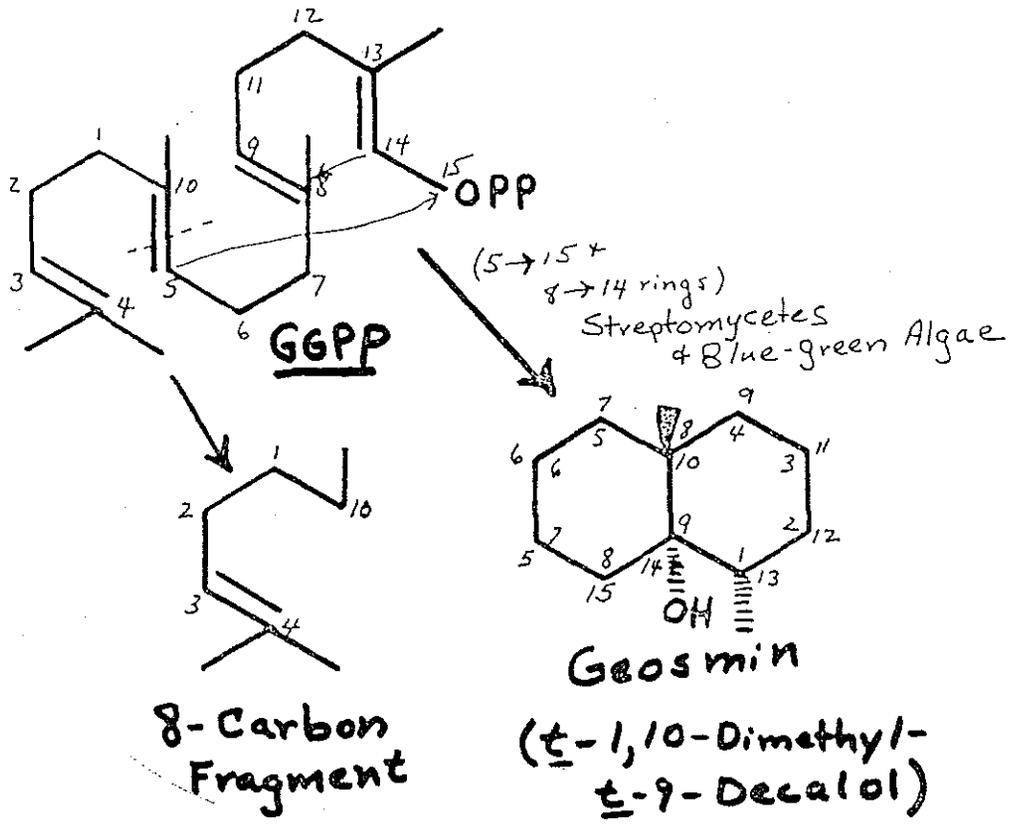
Geranylgeranyl
Pyrophosphate

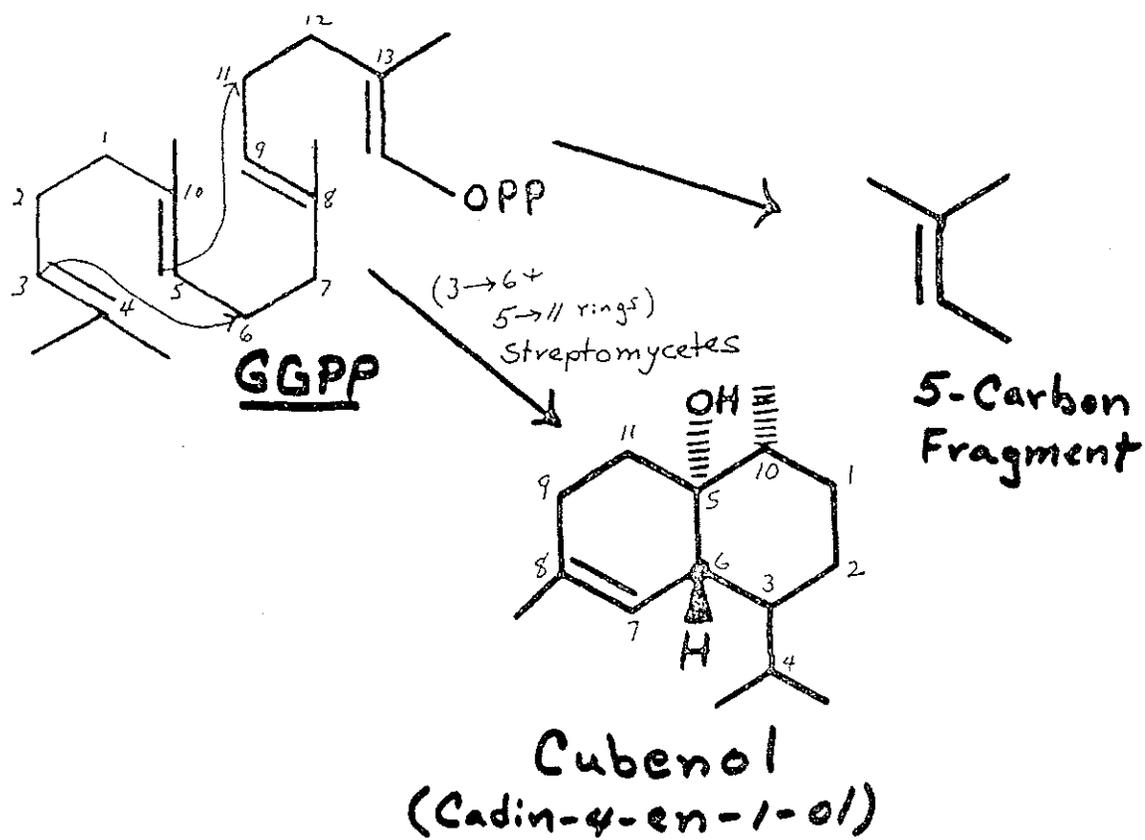


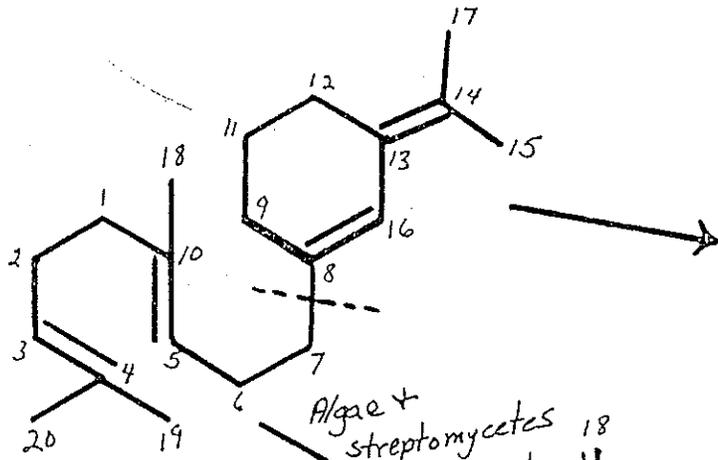
Key Intermediate



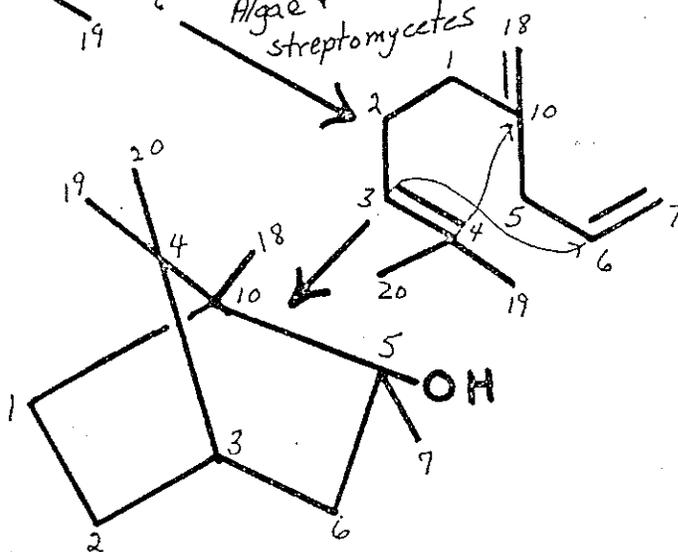
Dehydroabietic
Acid



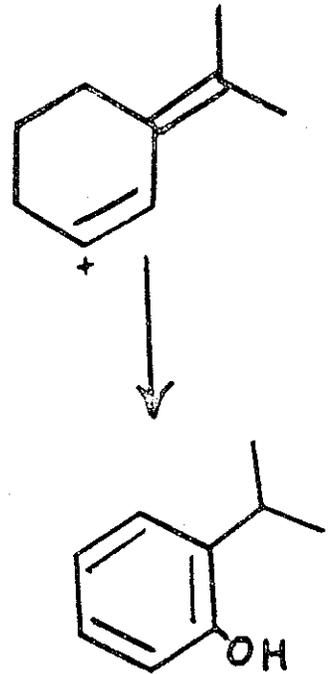




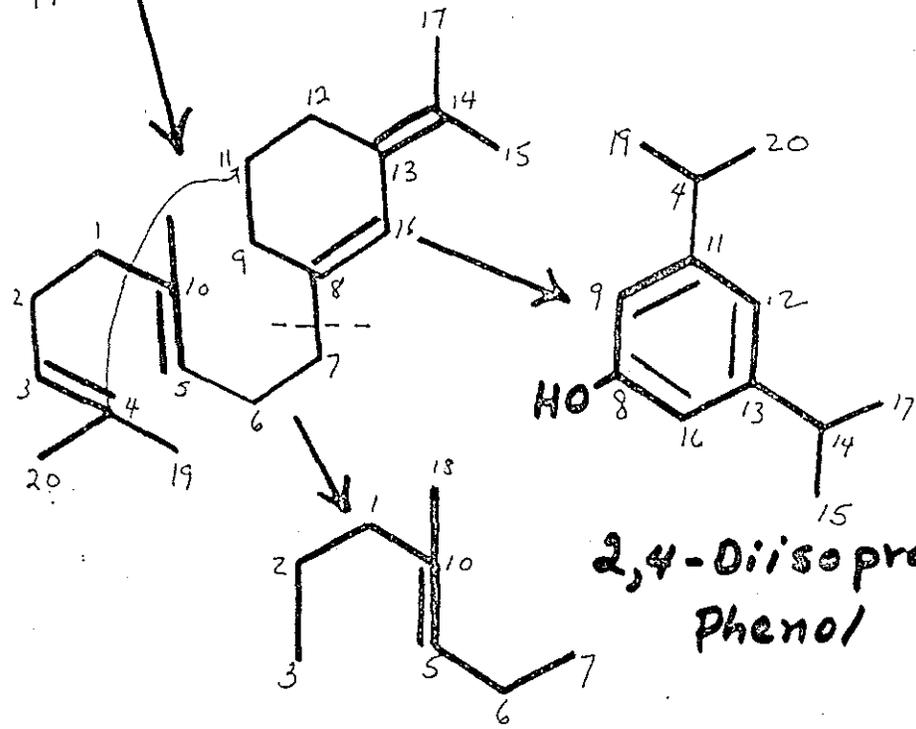
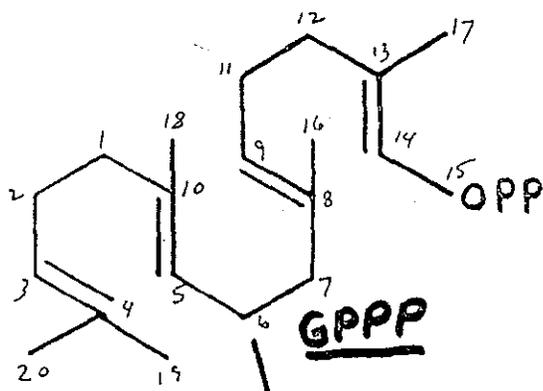
Algae + streptomycetes



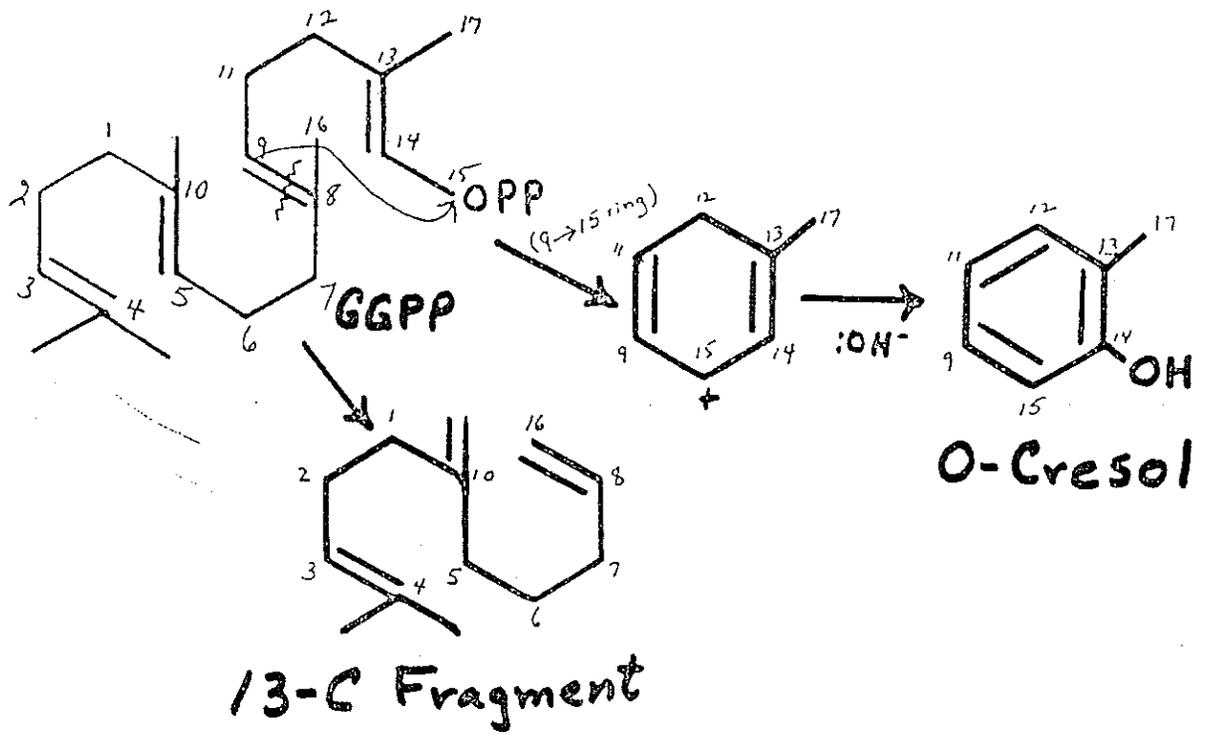
2-Methyl/isoborneol

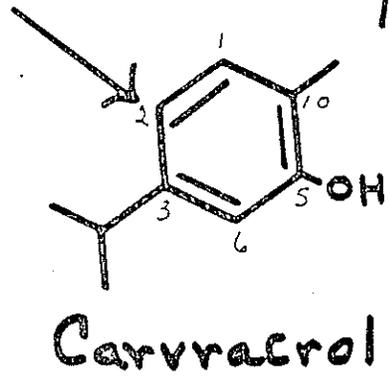
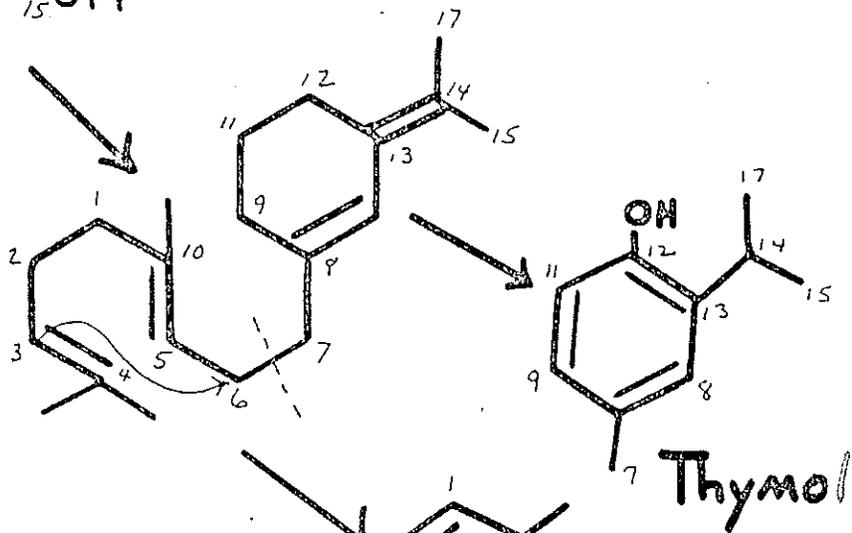
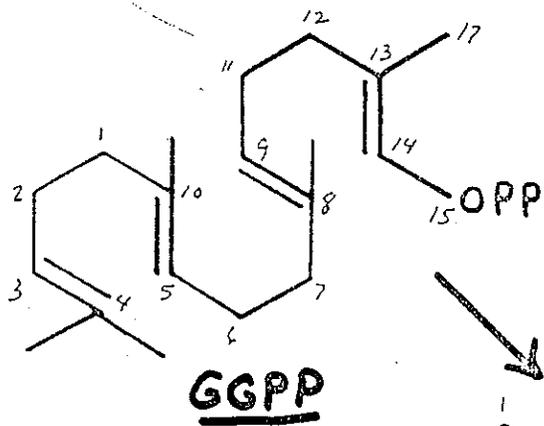


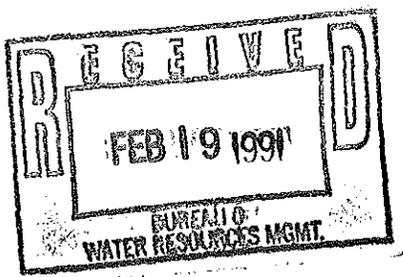
2-Isopropylphenol



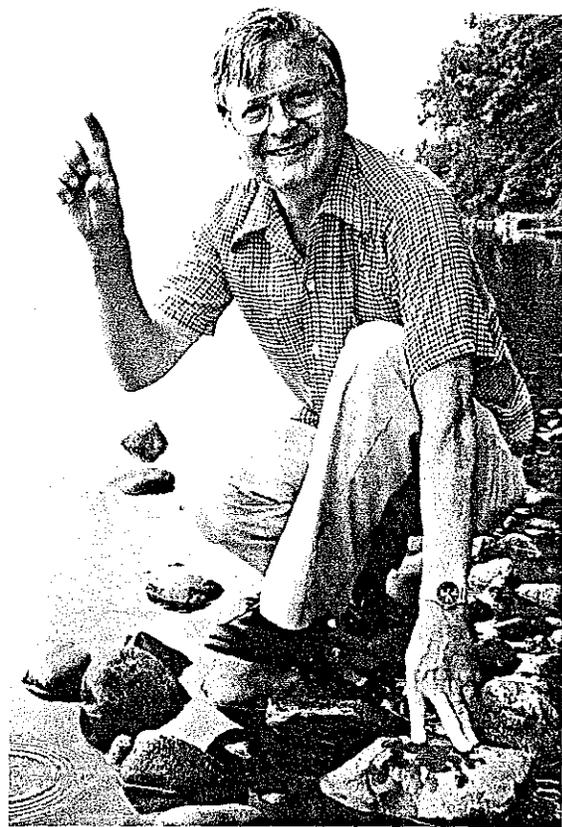
7-Carbon Fragment







Something fishy in the air



UNIVERSITY OF WISCONSIN NEWS AND INFORMATION SERVICE

John J. Magnuson.

You can't always trust your nose. Lake odors and fishy tastes that offend us don't necessarily harm us, but it's still worth clearing the air.

Natasha Kassulke

In 1990 I spent my first summer as a student in Madison away from home — IT STUNK! At my apartment on Langdon Street, I was bombarded with a septic smell from Lake Mendota. While vacationing, I turned up my nose at fish tainted with an off-flavor from a stretch of the Wisconsin River. Fishy odors from the lake didn't help my love life either. Instead of holding hands, my boyfriend and I held our noses.

These are funny memories as I enjoy an odorless snowfall, but looking ahead to the spring search for summer housing, I'm leery. I don't want another stinky summer by the lake. It's easy to joke about off-taste and odor problems in fish, lakes and rivers, but it's not a laughing matter.

Certain odors help us avoid perils and impending dangers, but what does the smell of rotting algae or the flavor of unpalatable fish really tell us? DNR water quality staff, lake and food experts from the University of

Wisconsin-Madison have joined forces to answer those questions. The group aims to show why "an ounce of prevention is worth a pound of cure" when controlling taste and odor in fish and water.

One of those with an eye and a nose for tracking changing lake conditions is John Magnuson, UW-Madison limnologist who has studied Dane County's Lake Mendota for 22 years. He says the lake odors that filled my apartment were caused by dying fish. The fish die-offs were caused by a complicated interaction among fish, water, temperature and algae.

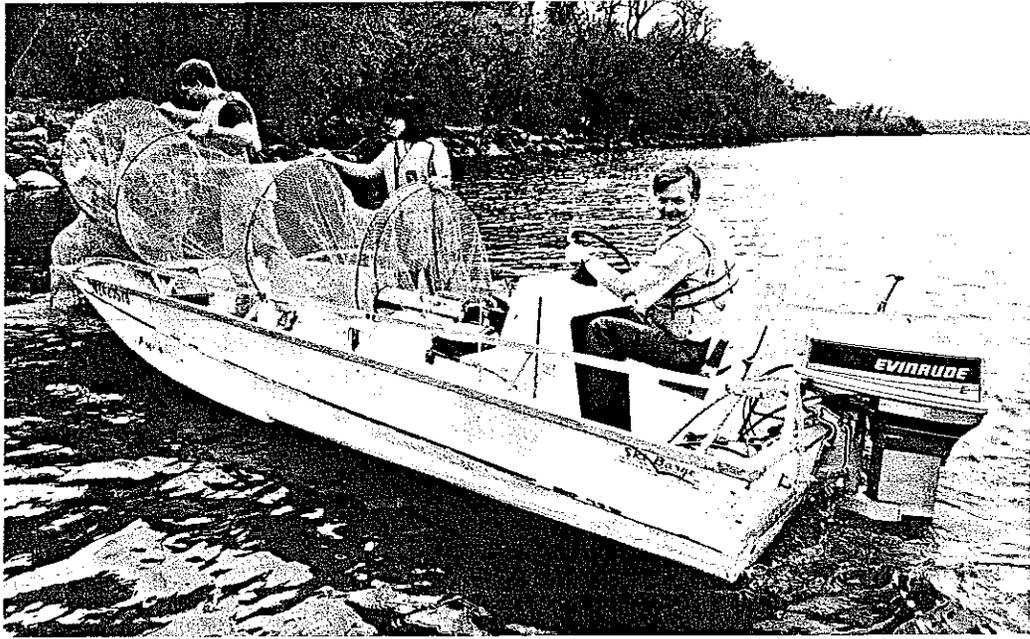
"Everyone gets old and dies," Magnuson says, smiling and pointing out the window of his second-floor office overlooking the lake. "Cisco are a major food source for other fish in the Madison lakes. Once the cisco grow bigger than minnow size, they don't face any other predators. They only die of old age, being caught by

anglers or during lake die-offs. In my opinion, die-offs happen when there is a very high density of fish."

He explained how these forage fish react to pressures from nearby cities and farms. Urban lakes receive lots of nutrients that encourage excessive algae and weed growth. As algae decay, bacteria deplete oxygen in cool, bottom waters. Bottom-dwelling fish like cisco are forced to move toward the surface where they die in warm waters. The dead, oily fish roll into shore and putrefy.

"We don't have massive fish mortality every year, just as we don't have massive blue-green algal blooms every summer," Magnuson continued. At moderate levels, aquatic plants are natural parts of the ecosystem, providing food, shelter, oxygen, spawning medium and nesting sites for fish and other aquatic organisms. Algae are typically viewed as nuisances rather than health threats.

However, some strains of blue-



COURTESY OF UNIVERSITY OF WISCONSIN SEA GRANT INSTITUTE

A playground and a living laboratory. Scientists have been studying the links among changing water chemistry, lake conditions, fisheries and the environment in Lake Mendota for more than 100 years. Professor Magnuson and University of Wisconsin Center for Limnology graduate students set fyke nets to periodically sample fish.

green algae produce toxins so they won't be eaten. These algae don't look or smell that different from harmless algae, but they act differently. When blue-green algae like *Microcystis*, *Nodularia*, *Coelosphaerium*, *Gloeotrichia*, *Aabaena*, and *Aphanizomenon* grow excessively, they excrete toxins that can kill animals, but only seem to cause rashes, eye irritations and stomach problems when people swallow them or swim through them.

There's nothing new about these cycles of algae blooms or die-offs on Lake Mendota. Trelease's 1889 paper, "The 'Working' of Madison Lakes" notes: "Every season a greenish-yellow scum occurs in greater or less quantity on Third and Fourth Lakes [Mendota and Monona], during the hot weather of summer after the water has been calm for a number of days in succession." Likening the lake to a vat of fermenting wine, some regarded this scum as evidence that the lake was "working." Using Trelease's definition, many Wisconsin waters are still working hard.

At times Mendota develops the unpleasant odors because it's a large lake (9,730 acres) surrounded by de-

velopment and agricultural counties. The damage starts in winter when runoff from 240 square miles of frozen terrain, roads, city streets, lawns and gardens drains into the lake. Nu-

trients also are released from lake sediments deposited during the days when household sewage was discharged into the water.

When the ice breaks up in early spring, sunshine, neutral pH waters and silicon get mixed in the water. Under optimal weather conditions, the mix forms big blooms of colonial algae called diatoms. The diatoms are ravenously grazed by water fleas and the bloom ceases rapidly. The water stays clear briefly in May, but looks are deceiving.

By late June a summer bloom of the blue-green algae, *Aphanizomenon flosaquae*, gives the water its characteristic green color. Other plants are shaded out and the algae predominates. In addition to eutrophying the lake, excess weeds and algae snag fish lines, interfere with commercial fish nets, and cause many of the odor problems and objection-

continued on page 33

(right) Natural cycles on Mendota are as clear as the water after ice melt. Daphnia feeding on diatoms flourish in early spring and produce unclouded waters. Summer blooms of blue-green algae are nourished by winter runoff from streets, fields and sediments.



COURTESY OF UNIVERSITY OF WISCONSIN SEA GRANT INSTITUTE

Flavor chemist Robert Lindsay examines how natural changes and human influences in lakes and rivers affect the taste and odors of fish caught from Wisconsin waters.

3-6712
3-2568

continued from page 10

able off-flavors in fish and other food.

Robert Lindsay, a UW-Madison food scientist and flavor chemist, has been studying off-flavors and taints in fish for more than 15 years.

"Little was known about off-flavors when we started looking at them," he remembers. "Industries [accused of causing odor problems] used to collect a bunch of fish. They'd host fish fries and say there was nothing wrong with the fish. Meanwhile, anglers were complaining about off-flavors in fish and the DNR started conducting taste tests."

To assess off-flavors more scientifically, Lindsay helped the DNR systematically collect fish, serve them to taste panels of 30 people and ask diners to describe the flavors.

"As lake water quality improves, the fish will have fresher flavors."

— Dave Stuibler

"By using panels, we got an average picture of the tastes of fish in certain waters," Lindsay recounted. "We plainly and conclusively described the fish flavors typically encountered in Wisconsin." By consensus, the taste panel documented off-flavors in fish from the Wisconsin River. Then Lindsay started investigating what caused these sporadic flavor problems.

Three distinct flavor taints were identified. The first, a kerosene-like or medicinal flavor, was caused by alkyl phenols, which entered the river from natural vegetation and industrial sources including wood fiber. In low concentrations, alkyl phenols didn't noticeably taint fish flesh, but in higher concentrations the compounds caused very offensive odors and flavors.

The second flavor, which was somewhat sulfurous, was caused by the thiophenol and thiocresol contained in paper mill effluents. Un-



COURTESY OF UNIVERSITY OF WISCONSIN SEA GRANT INSTITUTE

It's tricky business determining when fish have an off-flavor or odor. Perceptions vary. Food scientist David Stuibler has verified that people who regularly eat fish tainted with certain chemicals lose the ability to sense those odors and flavors.

like alkyl phenols, these compounds can cause off-flavors in low concentrations.

The third flavor, a foul-smelling earthy-must, was caused by the compounds geosmin and methyl isoborneol, which are produced by blue-green algae and other algae that colonize decaying vegetation. Lindsay describes the odor as a cattail odor: "The best way to simulate it is to leave your muddy clothes in a room and disappear for a while. When you return to the room, that is the smell of cattails."

The worst of all flavors, Lindsay notes, is a combination of all three off-flavors.

"Some people believe off-flavors are the natural flavors in fish," says David Stuibler, UW-Madison food scientist for 20 years.

His research verifies that people exposed to odors over time lose their sense of taste and smell.

"People who rely on their catches for most of the protein in their diet

consider every fish an edible game fish," Stuibler stated. "They've grown so used to the off-flavors that they don't recognize any difference."

"When you first bring fresh fish out of the water, it should smell like that seaweed odor you get standing at the end of a dock on a cool, damp day where the wind is blowing in from the lake," Stuibler says. "That's the normal flavor you should expect in high-quality fish."

Stuibler and Lindsay have been spending more time in recent years isolating the compounds that give fish good aroma. Research shows these fresh fish odors are similar to those found in melons and cucumbers. By isolating and highlighting these positive aromas, the scientists aim to improve the taste and shelf life of commercially-marketed fish.

The food scientists work with environmental specialists to improve water quality and fish habitat. Stuibler confidently predicts that as lake water quality improves, the fish sport an-

glers catch will have fresher flavors.

Tasty fish and fresh-smelling waters are part of DNR's vision for improved water quality. Aesthetic improvements naturally complement other actions to limit pollution discharges, slow lake eutrophication, stem toxics and monitor ecosystem health.

DNR and its research partners use a mix of biological, mechanical, chemical and regulatory tools to improve water quality. Parallel programs work on permanent solutions to keep agricultural wastes, city stormwater, lawn pesticides, garden fertilizers, soil and sediment from construction projects out of waterways.

"We intend to keep working with businesses and communities, on land and in the waterways," says Bruce Baker, director of DNR's Bureau of Water Resources Management. "Limiting sources that cause taste and odor problems complements our efforts to control toxics, organic and inorganic wastes in water."

Nevertheless, objectionable flavors and odors can be caused by such small amounts of compounds that it's

unlikely regulations would resolve all complaints.

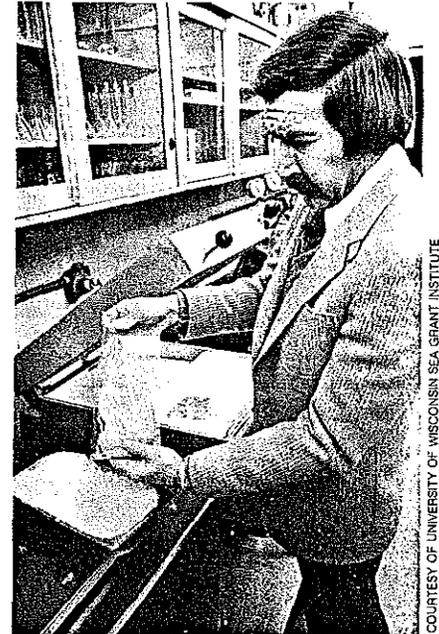
"You can't shut everything down to a zero discharge," Lindsay notes. "Monitoring industries along the rivers and lakes has lessened discharges or at least improved waste treatment

"Taste and odor problems in fish will be suppressed in the next few years, but they are certainly not going to go away."

— Robert Lindsay

prior to discharging. Taste and odor problems will be suppressed in the next few years, but they're certainly not going to go away."

Stuiber agrees, and he doesn't bemoan the fact that the days of pristine Wisconsin waters are over. "The first time you walk into an area and put



Robert Lindsay

COURTESY OF UNIVERSITY OF WISCONSIN SEA GRANT INSTITUTE

one foot in the water, you've changed it," he says. "Pristine' waters exist only in the eyes of the beholder."

Concerns about water pollution, taste and odor problems haven't dampened the DNR/UW team's enthusiasm for catching and cooking fish. In fact, their upbeat predictions have restored my faith in the future quality of both fish and surface waters. I'm looking forward to future springs when love, instead of decaying algae odor, will fill the air and the fish will be more tasty than ever. Now where did I put the address for that apartment by the lake?

Natasha Kassulke worked for DNR's Bureau of Water Resources Management while pursuing a journalism degree at Edgewood College in Madison.

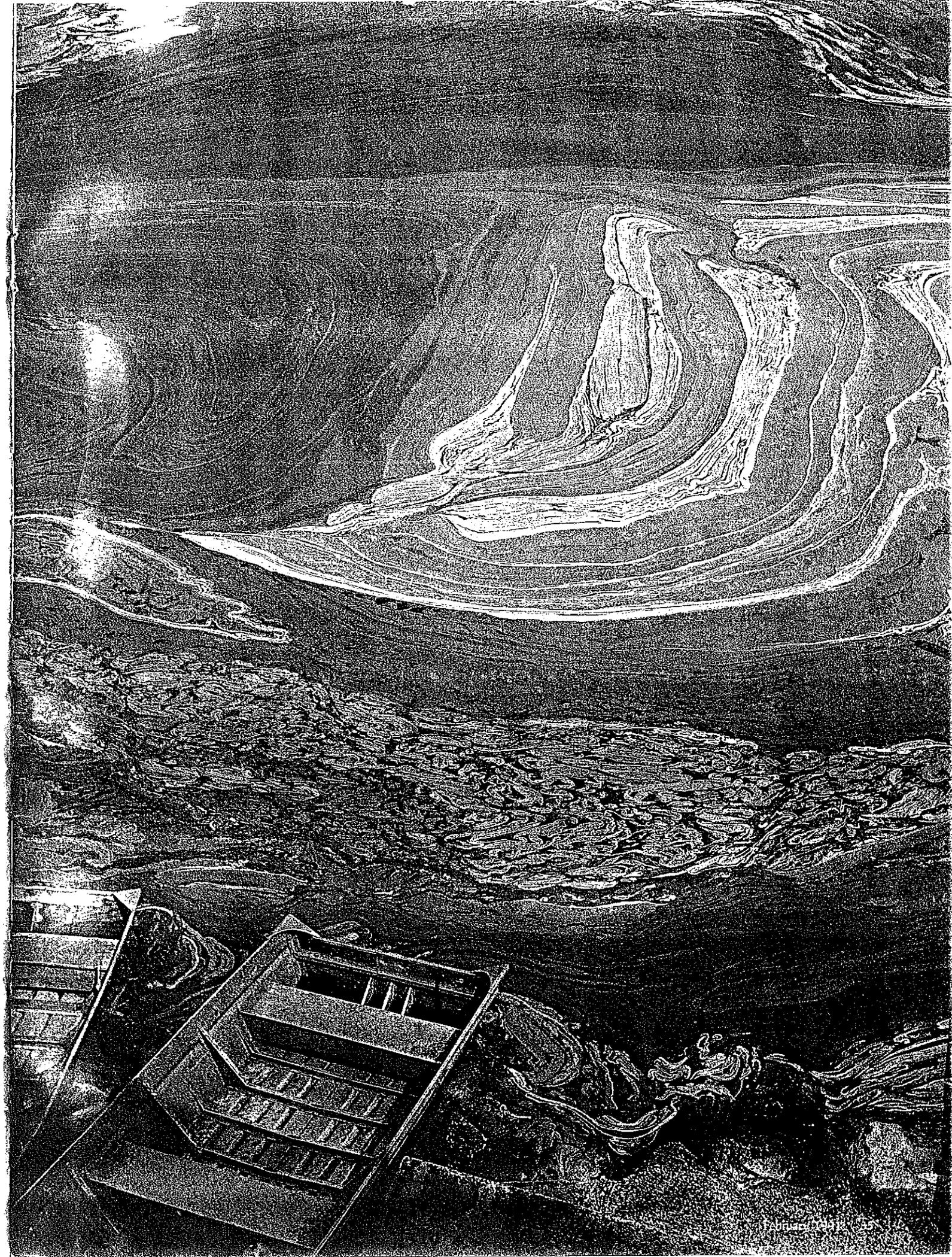


COURTESY OF UNIVERSITY OF WISCONSIN SEA GRANT INSTITUTE

Fish can absorb off-flavors from the waters where they live. Other odors taint fish during processing and merchandising. Food scientist Dave Stuiber works with commercial fishermen and processors to maintain and enhance fresh-caught flavors in fish.

(right) A blue-green algae bloom "working" on Lake Mendota.

UW CENTER FOR LIMNOLOGY



Wisconsin DNR correspondence regarding fish flavor tainting in Wisconsin waterbodies from 1984 - 1996

CORRESPONDENCE/MEMORANDUM

Date: January 11, 1984

File Ref: 3200

To: Bill Sonzogni - State Lab of Hygiene John Sullivan - NCD, Rhinelander
Doug Dube - State Lab of Hygiene Bob Martini - NCD, Rhinelander
Dave Dagenhardt - State Lab of Hygiene Lee Liebenstein - WRM/2
Robert Lindsey - UW Dept. of Food Science Jack Sullivan - WRM/2
Tim Heil - UW Dept. of Food Science F. Schraufnagel - TS/2

From: Jim St. Amant - WRM/2 JS

Subject: Analysis of Taste and Odor Compounds

This is to confirm our meeting arrangements which we discussed on January 9th. The meeting will take place on Tuesday, January 17, at 9:00 A.M. in Room 217 of the GEF 2 Building with a phone connection to Rhinelander.

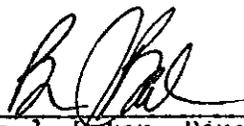
The purpose of this meeting is to discuss the extraction and analysis of taste and odor causing compounds in Wisconsin fish. We need to develop a coherent plan which will facilitate the investigation and alleviation of this annually recurring problem. The compounds which are the most probable causative agents and the proposed detection limits are listed below:

Diphenyl Oxide	1.0 ppm
Diisopropyl Phenols (2,4-; 2,6-; & 3,5-)	50.0 ppb
Thiophenol	15.0 ppb
Carvacrol	10 ppb
Isopropyl Phenols (3 & 4)	10 ppb

threshold

I look forward to meeting with you on the 17th.

JS:jm

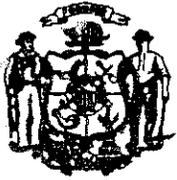


Bruce J. Baker, Director
Bureau of Water Resources Management

River Location	2-isopropylphenol	2,4-diisopropylphenol	2,5-diisopropylphenol	2,6-diisopropylphenol	3,5-diisopropylphenol	thymol	carvacrol	thiophenol	General Flavor Assessment
Below Prairie du Sac (March 14)	2.0	0	4.2	0	0	0.6	4.5	0	Alkyl phenol
Lake Wausau (April 18)	2.9	0	0.3	0	0	0.5	0	0	Strong alkyl phenol
Wisconsin Dells (April 26)	0.8	0	0	0	0	0	0	0	Good
Below Plover Dam (May 15)	8.2	1.5	0	0	3.0	0	13.0	0.9	Alkyl phenol & thiophenol
Prairie du Sac (June 6)	0.5	0	0	0	0	0	4.0	0	Muddy, earthy
Below Nekoosa (June 19)	0.8	0	0	0	0	0.6	0.8	0	Good
Below Petenwell (June 19)	1.3	0	0	0	0	0	1.5	0	Good

Std. dev. \pm 2% at 50 ppb; + 20% at 1 ppb. Based on laboratory spikes.

TABLE Concentrations of selected compounds in Wisconsin river walleye expressed in ppb (w/w).



State of Wisconsin \ DEPARTMENT OF NATURAL RESOURCES

Carroll D. Besadny, Secretary
Box 7921
Madison, Wisconsin 53707
TELEFAX NO. 608-267-3579
TDD NO. 608-267-6897

May 18, 1990

IN REPLY REFER TO: 3200

Robert C. Lindsay
Department of Food Science
1605 Linden Drive
Madison, WI 53706

SUBJECT: Off-flavors in Wisconsin River Fish:

Dear Bob:

Thank you for the complimentary copy of Tim Heil's thesis. It contains information that will be invaluable to us when dealing with questions concerning this matter of high public interest.

I've enclosed a few comments that we have been receiving regarding the unfavorable taste of Wisconsin fish that you may find interesting. I will keep you informed of any further developments that occur in this area.

Sincerely,

A handwritten signature in cursive script that reads 'Jim Amrhein'.

Jim Amrhein
Fish Contaminant Specialist
Bureau of Water Resources Management

Enclosures

CORRESPONDENCE/MEMORANDUM

Date:

File Ref:

To: Lee Lieberstein

From: Jean Umuth - Research

Subject: LWR fish



Hi Lee: 4 research employees have eaten Walleyes from fish caught below PDSAC Power Dam on 3/29/90. The were under 20" and no less than 14". All four of us had unfavorable reactions to the taste of the fish. Comments were: "Musty tasting", "inferior to Walleyes out of other bodies of water," "has a metallic flavor," and "I'll never eat those again".

I am interested to know if you have done any studies on PCB, and Mercury levels on fish on the lower Wisconsin River. Especially interested in gamefishes.

Thankyou

CORRESPONDENCE/MEMORANDUM

STATE OF WISCONSIN

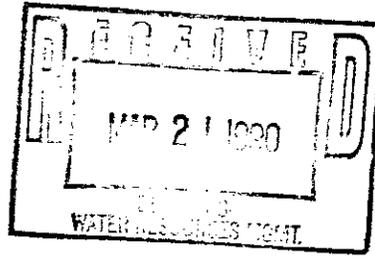
Date: 3/20/90

File Ref:

To: Lee Liebenstien

From: Jean Umuth

Subject: LWR. Fish taste/odor



Hi Lee:

Out of 349 anglers interviewed only 5 anglers had a comment about taste of LWR fish. The clerks do not actually ask the people for the comments but record any comments they make about the taste/odor. Comments are as follows.

- 1.) "Fish taste foul"
- 2.) "No unpleasant taste"
- 3.) "I keep few fish because they taste too bad"
- 4.) "fish here taste like Mosinee Smells, but I haven't eaten fish from here in several years"
- 5.) "Fish doesn't taste as bad as it did 5 years ago, but it is still a noticeable taste" - "especially in the Spring."

I'll keep you informed of any more comments. Sincerely, Jean Umuth

Lee

Received telephone report today from a Tom Kramer that Wis. R. fish, ^(walleye) from Nekoosa to Peterwell were 'bad' (taste/odor-wise) from early Apr thru early May. Referred him to Jack Z. and R. Detsen in WI Rapids Area to ask if there were other reports $\frac{1}{2}$ /on problems at the mills.

Dunham

CORRESPONDENCE/MEMORANDUM

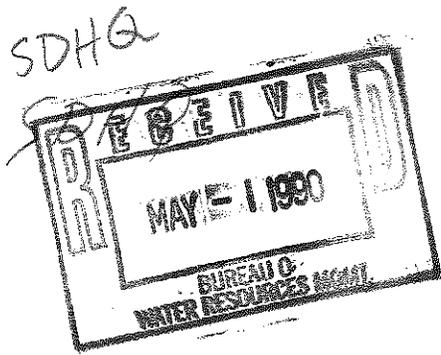
Date:

File Ref:

To: Lee Lieberstein

From: Jean Unmuth - Research

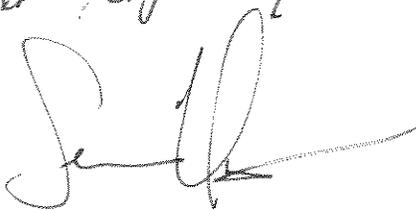
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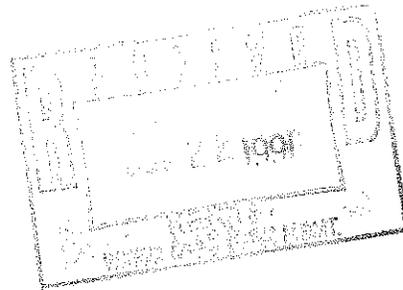
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Dunham

October 18, 1991

Mr. Paul Zugger
Surface Water Quality Division
DNR
P.O. Box 30028
Lansing, Michigan 48909



Dear Sir:

From friends of mine in Norway, Michigan, and Niagara, Wisconsin, I understand that the Champion Mill at Quinnisec, Michigan, has requested a variance in the fish taste testing limitations. I wish to voice my deepest concerns in this matter; in fact request quite the opposite.

Let me state some facts as I have heard them. First, Champion's other paper manufacturing facilities have already spoiled and polluted rivers in Florida and Tennessee. Their track record is apparent and obvious. They have chosen our Menominee River as their next conquest.

Do I have expertise in this area? Absolutely!! I have figuratively lived on this river since June of 1968. Up until mid 1986 my family had fished the river between the Norway Dam at Sturgeon Falls and the Pemene Dam and Quiver Falls eight miles east of Pembine, Wisconsin. Since Champion has been dumping its hardwood effluent at Piers Gorge, the Menominee River has been terribly degraded. The once clear water is now turbid in color. Ooze-like grayish slime covers much of the bottom, sand bars and structure. Floating plaques of repugnant debris are now very evident from mid July on. The water's olfactory qualities are acrid and oily and chemical in taste, and reek of paper mill odor. Sir, I do know odor!

Most offensive is the taste of the small mouth black bass. They are not fit for human consumption. Each trip this year I have taken two fish home and tried to eat them. In fact the cat will not eat them and treats the fish like the 'logs' in her sand box.

Mr. Paul Zugger

Page two

For five years I have been writing your offices in Lansing and Marquette, EPA in Chicago, Green Bay and Madison, Wisconsin DNR offices, and state and federal politicians. I gather that one voice from the stern of an Old Town canoe is inaudible compared to the clout of Champion International, One Champion Plaza, Stamford, Connecticut.

Do not let this monster destroy our primitive, pristine waterway.

I wish to testify against them.

Sincerely,

A handwritten signature in cursive script, appearing to read "H.S. Kalinka" followed by a flourish.

H.S. Kalinka, D.V.M.
307 W. Breed Street
Chilton, Wis. 53014

cc: Delbert Rector, Director Mi. DNR
William Taft
Jack Rydquist
Jim Amrhein, Wis. DNR
Rich Kinsella, Niagara, Wi.
Sue Doersch, Ironwood, Mi.
Donna Hayes, Norway, Mi.

MICHIGAN DEPARTMENT OF NATURAL RESOURCES

INTEROFFICE COMMUNICATION

→ TO: DUANE SCHUETZELZ WR/Z
 FROM: TIM DOELGER —

October 28, 1991

TO: Jack Rydquist
 Marquette District Office
 Surface Water Quality Division

DUANE, OF SPECIAL NOTE - LAST PARAGRAPH -
 I'VE BEEN TALKING TO THEM & BOB
 BEHRENS ABOUT ASKING FOR A
 TASTE TEST IN THE NEXT PERM.

FROM: William H. Taft and William Creal
 Great Lakes and Environmental Assessment Section
 Surface Water Quality Division

SUBJECT: Champion International-Quinnesec Mill
 Fish Taste Impairment Study (#2)
 MI0042170

We have reviewed the Fish Taste Impairment Study #2 submitted on July 30, 1991 to Marquette District Office as specified in Special Condition #5 of the subject facility's NPDES permit. The test was run using effluent dilutions of 2.2% and 4.4%. The Instream Waste Concentration (IWC) is 4.4%. The results of the study indicate that there was a statistical difference between the 2.2% dilution and the blind control at the 98% level of significance and a statistical difference between the 4.4% dilution and the blind control at the 94% level of significance. This test demonstrates that the facility's effluent has the reasonable potential to impair fish flesh taste.

This is the fourth taste test conducted since 1985 involving fish taint. The first tests in 1985 involved native Northern Pike and Walleye from the Menominee River downstream of the Champion and Niagara paper mills. In addition, IN SITU caged perch studies were conducted from 5 separate river locations between the Henry Ford Dam and the Chalk Hills Dam. Native walleyes and northern pike from the Champion intake pool demonstrated significantly lower overall flavor acceptability as compared to the other stream locations. The IN SITU caged perch tests showed no significant differences between sites.

The last three fish flavor studies (Spring & Fall, 1989 and Spring, 1991) were follow up studies conducted as flow through effluent tests using the ASTM protocol. In every instance, the Champion effluent has demonstrated a statistically significant difference at effluent concentrations less than or equal to the IWC. These tests were conducted to simulate worse case river conditions (i.e. Q710 low flow and maximum effluent flows).

We conclude, as a result of the recently submitted fish taste data that the Champion effluent has a reasonable potential to cause instream fish flavor impairment under Special Condition #5,

Section C., of the NPDES permit. However, the tests to date have not identified the cause of the impairment or the contribution to instream taste impairment by in situ river factors or other industrial dischargers. To address these issues, we therefore recommend that Champion be notified in writing under Section C of Special Condition #5 that, within 90 days of notification, Champion is required to submit a study plan to address potential fish flavor impairment by their discharge; and also evaluate enhancement factors in the river.

The study plan required should include a three phased approach: Phase 1 - information gathering and compound identification; Phase 2 - quantification and compound isolation; Phase 3 - treatment evaluation and process modification. Specific tasks and completion dates should also be included as part of the study plan.

Important elements to be included in Phase 1 are as follows:

- 1) Complete fish tissue and effluent scans that address all known and suspected tainting compounds including volatiles, dissolved gases, and organics;
- 2) A list, by process, of raw materials, trace contaminants of raw materials, trade name formulations, intermediates, intended byproducts, unintended byproducts, products used or produced in or incidental to facility processes and present in wastewater;
- 3) A complete literature review of the tainting and chemical transformation data available for the list of materials in #1 and #2 above.;
- 4) Characterize physical and chemical properties of wastestream compounds for factors such as degradability, filterability, solubility, volatility, etc;
- 5) Additional fish tastes tests to better define and identify potential sources of impairment, including any proposed modifications of such tests; and
- 6) River studies to address factors which may enhance flavor impairment in native fish.

Phase 2 should include elements that address the isolation and measurement of the suspected chemical tainting agents identified in Phase 1. Phase 3 should include evaluations of appropriate treatment technology and/or process modifications that will eliminate or appropriately reduce fish tainting in controlled taste tests approved by the department. The elements recommended in this three phased approach are to be used as a general guide for submitting an approvable study plan.

In addition, we have been discussing with the State of Wisconsin the possible influence of Niagara of Wisconsin Paper Company (N.O.W.) on the fish flavor impairment issue. The N.O.W. fish taint results from the spring 1989 study are regarded as suspect because the paper mill was shut down in the middle of the study, and study methods did not strictly follow ASTM methods. Until this issue is settled, questions about the N.O.W. facility and it's impact on the Menominee River will be in doubt. This

information will help isolate the other major source discharger which has the capability to directly influence fish tainting in the Menominee River. No final determination can be made until this information is available.

cc: GLEAS Files

STATE OF WISCONSIN
Prepared By MI, DNR

Meeting Summary

Michigan and Wisconsin Department of Natural Resources

Fish Tainting in the Menominee River

June 8 , 1988

On June 8, 1988, a meeting was held between representatives of the Wisconsin and Michigan Departments of Natural Resources (DNR) to discuss tainting of fish in the Menominee River in the vicinity of Niagara of Wisconsin and Champion International Corporation Paper Companies. The meeting was held in Marinette, Wisconsin. A list of attendees is attached

For the last two years complaints of poor tasting fish, primarily walleye, have been reported to the Michigan and Wisconsin DNRs. The greatest number of complaints have been received during early spring. The majority of poor tasting fish have been caught from a portion of the Menominee River called the Wisconsin slough, located just upstream of the confluence of the Menominee and Sturgeon Rivers and downstream of Niagara and Champion paper companies. The tainted fish have been reported to be inedible due to a variety of disagreeable odors and/or tastes. No taint complaints have been received regarding fish caught above the Little Quinnesec Falls Dam, a barrier to upstream fish movement from the Wisconsin slough and the Niagara and Champion mills.

As an NPDES permit condition Champion International Corporation conducted taste and odor studies in the vicinity of the mill using caged and indigenous fish (IPC, 1987). The results of the October, 1986 study showed that there was significant off-flavor of caged perch below the Champion process waste outfall compared to above the discharge. During the fall 1985 and 1986, and spring 1986 studies, there was significant taste and odor impairment of indigenous northern pike and walleye collected from below Niagara of Wisconsin and Champion International compared to above the Little Quinnesec Falls Dam. The study design was such that the source of the taint producing substance(s) could not be identified.

In an attempt to identify the source or sources of taint producing substances the Wisconsin and Michigan DNRs have agreed that both Niagara of Wisconsin and Champion International need to conduct fish taint studies that will isolate the effects of their effluents on fish taste and odor. This will be best achieved using the laboratory study method described in ASTM procedure D3696-78, Standard Practice for Evaluating an Effluent for Flavor Impairment to Fish Flesh. Test fish, preferably walleye, would be exposed in a flow through system to the final process waste effluent of both facilities at the permitted instream waste concentration after mix with 25% of the Menominee River 7Q10 flow. One test per facility would be considered adequate since the effluent from both facilities is consistent year round. Potential sources for walleye of an adequate size for testing included commercial hatcheries or a local lake.

Following an appropriate exposure period the taste and odor characteristics of the test fish would be evaluated by a panel. the resulting data would be statistically analyzed to evaluate whether either facility is discharging taint producing substances to the Menominee River in significant amounts.

In addition to the laboratory method described above, a number of other ideas were suggested to identify the source(s) and types of taint producing substances. These included:

- 1) analysis of the Niagara and Champion effluents during the laboratory study for known taint producing compounds.
- 2) analyze the tissue of fish exposed in the laboratory for known taint producing compounds.
- 3) analyze benthic macroinvertebrates in the vicinity of the Niagara and Champion discharge for known taint producing compounds.
- 4) analyze the tissue of indigenous fish for known taint producing compounds.

Wisconsin Department of Natural Resources (WDNR) agreed to look into the EPA's approach to setting chemical specific taste and odor effluent limitations. Mr. Mike Bartz, WDNR Conservation Warden, and Vern Nurenberg, MDNR Fisheries, agreed to provide the meeting attendees with a summary of Menominee River fish taint complaints to help better identify potential tainting sources and the downstream extent of taint complaints.

MDNR agreed to draft a letter to Niagara of Wisconsin and Champion International requesting the ASTM fish taste and odor studies.

Environmental, Health, and Safety
P.O. Box 191
Marquette, Michigan 49870-0191
313 773-3256



April 14, 1993

Jack Rydquist
District Supervisor
Surface Water Quality Division
1990 U.S. 41 South
Marquette, MI 49855

Dear Mr. Rydquist:

This letter is to advise you of continuing progress made at Champion International Corporation's Quinnesec mill to minimize upstream waste loading from the mill's processes and to further improve its wastewater treatment plant effluent quality.

As you recall, Champion's current NPDES permit required the mill to conduct two fish flavor impairment studies. These studies were conducted November 1989 and April 1991. Results from these studies indicated a statistically significant difference between the blind control (fish exposed to river water only) and fish exposed to the mill's effluent. Since completion of these studies, Champion has implemented programs to minimize sewer losses and optimize wastewater treatment operations. Detailed below is a summary of the action steps implemented:

Oxygen Delignification

Since implementation of oxygen delignification in May 1990, organic loadings from the mill's pulping process have been reduced significantly. This enables the mill to dramatically reduce molecular chlorine needed to maintain pulp quality targets, thereby minimizing the potential formation of chlorinated organic compounds. Also, the reduction in organic loading from the pulping process is benefiting overall operations of the mill's wastewater treatment facility.

Chlorine Dioxide Substitution

The mill was originally designed to deliver chlorine dioxide substitution levels of 10% to the bleaching process. Implementation of oxygen delignification in the mill's pulping process and various state-of-the-art bleach plant equipment and process changes have allowed the mill to increase chlorine dioxide substitution rates beyond the mill's original design.

These process changes and modifications have enabled the mill to reach chlorine dioxide substitution rates up to 60%. Increased chlorine dioxide substitution levels have further reduced molecular chlorine usage.

Sewer Loss Minimization

A comprehensive, three phased program was initiated early in 1992 in order to reduce loadings to the mill's wastewater treatment facility. Each phase of the sewer loss reduction program is summarized below:

Phase 1: Spill Collection System Improvements

A task force addressing Pulp Mill and Recovery and Utility (R&U) spill collection sumps was organized in February 1992. The original design of the mill included nine sumps located throughout the facility. The sumps were intended to collect small leaks or spills such as packing leaks, expansion joint blow outs, and various process overflows. Problems identified with optimum operation of the sumps included: sump pluggage, line pluggage, product contamination (quality), temperature, and consistency (process parameters). A project to correct these operational problems was initiated in March and completed in May 1992. This project was successful in eliminating many of the problems which had previously reduced the effectiveness of the sumps. Losses of various process liquors have been substantially reduced as a result of this project. This has ultimately resulted in reduced loadings to the wastewater treatment facility.

Phase 2: Q-41 Paper Machine Sewer Loss Reduction

The Quinnesec mill was originally constructed as a non-integrated Kraft pulp manufacturing facility. A coated high quality paper machine (Q-41) was added in 1990 and began operation in October 1990. Waste loadings to the wastewater treatment facility initially increased with the addition of Q-41. Many operational and "start-up" related problems were experienced during the first 12-18 months of operation resulting in higher than normal sewer losses. Since January 1992 to date, sewer losses from Q-41 have been reduced by over 60 %. This reduction is attributed to various sewer loss reduction programs implemented in the Q-41 complex as well as increased runnability of the machine after "start-up". This has ultimately resulted in reduced loadings to the wastewater treatment facility.

Phase 3: Enhanced Black Liquor Recovery Project

A task force addressing black liquor losses from the brown stock washing system was organized in September 1992. The objective of the task force was to eliminate sewerage of black liquor contaminated filtrate from the brown stock cleaners. A project was developed to install and collect the 5th stage cleaner reject flow and the 2nd stage reject cleaner reject flow, then return the filtrate to the brown stock washer system rather than discharging it to the Pulp Mill sewer. This project has an estimated completion date of May 1, 1993. Champion intends to complete this project even though the results of the December 1992 and February 1993 fish flavor impairment studies indicate that no statistically significant flavor impairment is being created by Champion. After completion, this project will ultimately reduce loadings to the waste treatment facility.

Wastewater Treatment Plant Optimization

The action steps detailed above have dramatically improved overall wastewater treatment plant performance. Since January 1992, biochemical oxygen demand and total suspended solids loadings to the wastewater treatment facility have been decreased by over 50%. These significant reductions have allowed the mill to optimize wastewater treatment operations, which is reflected in greatly improved effluent quality.

In summary, Champion has taken aggressive steps to minimize sewer losses and enhance its effluent quality. The positive results of the fish taste impairment studies conducted December 18, 1992 and February 17, 1993, are clearly indicative of Champion's success and demonstrates the effectiveness of the programs outlined in this letter. Even though it is very clear that Champion's effluent is not impacting fish flavor in the Menominee River, Champion will continue to look for opportunities to minimize loadings to its wastewater treatment facility, which will further enhance the quality of effluent discharged to the river.

If you have any questions, please call me at (906) 779-3444.

Sincerely,

Walter Blair
Walter Blair
Environmental Supervisor

cc: Ed Clem
Steve List
Tom Siegrist

Jeff Hearn
Rich Menard *

psn\dnr\2198

APPENDIX B

Table 1. Raw Panelists Scores for Fish Flavor Evaluation, Modified ASTM Procedure, December 18, 1992.

Panelist	Blind control ¹	2.2% Effluent ²	4.4% Effluent ³
1	+2	-1	-1
2	-1	-2	-2
3	-1	-1	0
4	+1	-2	-3
5	-1	2 ⁴	-2
6	+3	+2	+1
7	+1	-2	+3
8	+1	+2	0
9	-1	-2	-3
10	-2	-3	+2
11	+1	+2	+3
12	+3	+2	-1
13	-2	0	+1
14	-1	0	+1
15	+1	0	-1
16	+3	-1	0
17	0	+1	-3
18	+3	+2	-1
19	+1	-1	+2
20	+2	+1	-2
21	+1	+3	+2
22	-1	+1	-2
23	-1	-2	0
24	+2	-1	+1
25	-1	+2	+2
Total Score	+13	+2	-3
Average Score	+ .52	+ .08	- .12

¹Random Number 748²Random Number 442³Random Number 661⁴Panelist did not place a positive sign in front of score

Table 2. Ranking Scores for Fish Flavor Tasting Panel, Modified ASTM, December 18, 1992.

Panelist No.	Blind Control	2.2%	4.4%
1	1	2.5	2.5
2	1	2.5	2.5
3	2.5	2.5	1
4	1	2	3
5	2	1	3
6	1	2	3
7	2	3	1
8	2	1	3
9	1	2	3
10	2	3	1
11	3	2	1
12	1	2	3
13	3	2	1
14	3	2	1
15	1	2	3
16	1	3	2
17	2	1	3
18	1	2	3
19	2	3	1
20	1	2	3
21	3	1	2
22	2	1	3
23	2	3	1
24	1	3	2
25	3	1.5	1.5
Total	44.5	52.0	53.5
Difference from Total		7.5	9.0
Critical values from Table, p = 0.01 is 18; p = 0.05 is 14		lower than critical difference value	lower than critical difference value

APPENDIX B

Table 1. Raw Panelists Scores for Fish Flavor Evaluation of Walleye Fillets from fish exposed to 2.2 and 4.4% Effluent using the Modified ASTM Procedure, February 17, 1993.

Panelist	Blind control ¹	2.2% Effluent ²	4.4% Effluent ³
1	+3	0	+1
2	+1	-2	-1
3	0	+1	+2
4	+3	-1	+2
5	-2	-1	+2
6	+1	-1	-2
7	+1	+1	+2
8	0	-2	+2
9	+2	+1	-1
10	-1	+2	+1
11	+3	+2	-1
12	0	-1	+2
13	+2	+1	+3
14	-1	+1	+2
15	+2	+2	+1
16	-2	+1	0
17	+2	+1	-2
18	+1	-2	+2
19	+2	-2	-1
20	+1	+2	+1
21	0	+1	-1
22	-2	+2	-1
23	+2	+1	-1
24	+2	-3	+2
25	+1	+1	+2
Total Score	+21	+5	+16
Average Score	+.84	+.20	+0.64

¹Random Number 862²Random Number 989³Random Number 229

APPENDIX B

Table 2. Ranking Scores for Fish Flavor Tasting Panel for Walleye Fillets Obtained From Fish Exposed to 2.2 and 4.4% Effluent Using the Modified ASTM, February 17, 1993.

Panelist No.	Blind Control	2.2%	4.4%
1	1	3	2
2	1	3	2
3	3	2	1
4	1	3	2
5	3	2	1
6	1	2	3
7	2.5	2.5	1
8	2	3	1
9	1	2	3
10	3	1	2
11	1	2	3
12	2	3	1
13	2	3	1
14	3	2	1
15	1.5	1.5	3
16	3	1	2
17	1	2	3
18	2	3	1
19	1	3	2
20	2.5	1	2.5
21	2	1	3
22	3	1	2
23	1	2	3
24	1.5	3	1.5
25	2.5	2.5	1.0
Total	47.5	54.5	48.0
Difference from Total		7.0	0.5

Niagara of Wisconsin, Fish Taste Study

Contacts: Bill Taft, MDNR 517-335-4205
Gary Schnicky, MDNR 906-875-6622
Richard Menard, Champion 906-779-3642

1. Results of ASTM Tests post CIC process changes

- a. February 1992 test results indicated a taste impairment in rainbow trout, walleye were not available for study.
- b. Since 1992 February test, 2 more ASTM tests were done; these tests showed no taste impairment. The results were well within the appropriate Confidence levels of 95%.

*Bill Taft will send results. An April 4, 1993 memo from Wally Blair to Jack Ryquist summarizes the results of these 2 tests.

- c. After the February 1992 results CIC incorporated on a pollution prevention/process changes evaluation plan.

2. CIC Evaluation/Changes

- a. CIC incorporated a complete overhaul of sewage pumps, less waste to treatment system and more recycling, diluted effluent
- b. oxygen deliquification
- c. Chlorine reduction; reduce chlorine by 65%
- d. There have been D.O. problems @ the NOW mill; Bruce Oman should be contacted
- e. There have not been recent complaints to my knowledge. However I only talk to 2 people Tom Thuemler WDNR & Gary Schnicky MDNR.
- f. Since the NOW mill has had D.O. problems. It has been stated that BOD can play a big role in fish tainting. Robert Lindsay U.W. Madison Food Science should be asked this question.

*Bruce Oman should be contacted on the D.O. problem.

- g. It should also be noted that NOW's effluent feeds into CIC's intake pipes

3. If Study @ NOW proves Fish Taste Impairment

- a. NOW should run the modified ASTM test method. It is less subjective, and is the test CIC ran. The scoring is different instead of 0-4, it's defined as -3, -2, -1, 0, +1, +2, +3.
- b. EA Science Chicago was the consulting firm that CIC first used. Later CIC utilized the help of MI State University, Janice Heart PhD (517) 322-0614.

- c. CIC ran 4 ASTM Test Studies, cost \$50K-60K.
 - d. If NOW proves to be a problem towards fish taste impairment testing, greater than 5% of the time. Then NOW should submit a taint identification and reduction evaluation study plan along with a pollution prevention plan such as CIC (see memo) and 3 phase study plan on ASTM method 3696.
4. Walleye Test, NOW & CIC 1989
- a. This test was not incorporated into the WPDES permit. The statistical analysis/results were poor to define. See memo Eugene Lange and Robert Lindsay.
 - b. Since the effluent was stored prior to testing this is not valid. No documentation on Department approval to hold effluent.

*The District should be contacted on this.

*Also contact District on recent complaints, besides Tom Thuemler

v:\perm\wr9nowmt.bjg



George E. Meyer
Secretary

State of Wisconsin \ DEPARTMENT OF NATURAL RESOURCES

101 South Webster Street
Box 7921
Madison, Wisconsin 53707
TELEPHONE 608-266-2621
TELEFAX 608-267-3579
TDD 608-267-6897

April 1, 1993

Gerald Saalfeld
Surface Water Quality Division
Michigan Department of Natural Resources
P.O. Box 30028
Lansing, MI 48909

Dear Mr. Saalfeld:

Attached is a DRAFT permit for the Niagara of Wisconsin Paper Corporation. The Wisconsin Department of Natural Resources has incorporated the recommendation to include the fish taint study in the WPDES permit. I have tried to be as consistent as possible in drafting the language for N.O.W. to perform this study. Please review. The pages of particular concern are page 27 of the report and also the cover memo.

If you have concerns or questions please call me at (608) 266-3484.

Sincerely

Bonnie J. Goodweiler
Surface Water Standards Unit
Bureau of Water Resources Management

cc: Duane Schuettpelz - WR/2
Jack Sullivan - WR/2
→ Jim Amrhein - WR/2
Lee Liebenstein - WR/2

DATE: March 31, 1993

TO: Mike Witt - WW/2

FROM: Duane Schuettpelz - WR/2

SUBJECT: Water Quality Based Effluent Limitations for Niagara of Wisconsin Paper Corporation (WI-0000752)

DRAFT

Subject to revision
Do not cite or quote

This is in response to your request for an evaluation of water quality based effluent limitations using chapters NR 105 and 106, Wis. Adm. Code, for Niagara of Wisconsin Paper Company discharging to the Menominee River in Niagara.

Based on our review, the following recommendations are made on a chemical-specific basis, using a discharge rate of 5.4 mgd.

Table 1 lists those substances which are recommended for inclusion in the WPDES permit along with the indicated limits accompanied by regular monitoring and compliance schedules where appropriate (the footnotes are summarized following Table 3):

Table 1 - Outfall 017

<u>Substance</u>	<u>Daily Maximum (ug/L)</u>	<u>Weekly Average (lbs/d)</u>	<u>Monthly Average (mg/L)</u>	<u>Test Method (s)</u>	<u>Foot- notes</u>
Pentachlorophenol	11.0 (185 lbs/yr)			EPA # 604	
Phosphorus	(Monitoring only)		1.0		2
Aluminum	1500 (24,600 lbs/yr)			EPA # 202.2	1

Table 2 lists those substances which are recommended for inclusion in the WPDES permit along with the indicated limits and a requirement to perform one test within 12 months of reissuance or modification, using the indicated EPA test method or an approved test method providing a level of detection of 1/5 the indicated limit or lower. For each substance listed in Table 2, the limit(s) may be removed from the permit following that initial test if that substance either is not detected using the indicated test method or is not detected at a level of detection equal to or less than 1/5 the indicated limit (the footnotes are summarized following Table 3):

Table 2 - Outfall 017

(No recommendations pursuant to this section at this time)

Table 3 lists those substances which are recommended for inclusion in the WPDES permit with a requirement to perform two tests within 12 months of permit reissuance or modification. The footnotes are summarized following this table:

Table 3 - Outfall 017

The following substances have been identified as anthropogenic substances that cause fish taste impairment and are recommended to be monitored for in Niagara Paper's effluent.

Substance

2-isopropylphenol
3-isopropylphenol
2,4-diisopropylphenol
thiophenol
2-methyl-5 isopropylphenol (carvacrol)
4-isopropylphenol
2,5-diisopropylphenol
2,6-diisopropylphenol
3,5-diisopropylphenol
p-thiocresol
2-isopropyl-5-methylphenol (thymol)
m-cresol

RECOMMENDATION: Two additional tests are recommended within 12 months of permit reissuance or modification, using EPA test method 604, SW846-8040A or 8270B, depending on the specific circumstances and available equipment. The limits of detection for these substances should be in the range of 0.01 to 0.1 ug/L. Niagara Paper should submit these to the Department for evaluation.

In addition Niagara Paper, within 6 months of permit reissuance, shall submit an approval fish flavor impairment plan outlining specific testing and reporting procedures to the Department for approval. The plan shall include two fish impairment tests using effluent from outfall 017. The Department will have 90 days to approve the study plan. The test procedures shall follow the modified ASTM Designation: D3696-89 laboratory study "Standard Practice for Evaluating an Effluent for Flavor Impairment to Fish Flesh". Test effluent concentrations shall be a ----- % of the effluent. The study plan shall be consistent, in all applications, as the "Fish Taste Study Plan for Champion International Corporation Quinnesec Mill Quinnesec, Michigan", dated February 1992.

The final report on the test results shall be submitted to the Department within 90 days after completion of each test.

The Department will review the flavor impairment data to determine if significant fish flavor impairment has occurred. If the significant impairment occurs the Department may include appropriate measures to identify and eliminate the effluent constituents causing flavor impairment, additional study(ies) to show the effectiveness of the control measures.

Footnotes:

- 1 - Measurement in the "total recoverable" form is acceptable.
- 2 - The Administrative Rule NR 217 entitled "Effluent Standards for Phosphorus" is now in affect. An effluent limitation equal to 1 mg/L total phosphorus as a monthly average shall apply in cases the discharge of wastewater from all outfalls of a facility other than those subject to ch. NR 210 contains a cumulative total of more than 60 pounds of total phosphorus per month, unless an alternative limitation is provided. The Department shall determine if a permittee is discharging more than the applicable threshold value by examining the available data or requiring monitoring.

Additionally, Table 4 lists those substances which are recommended for inclusion in the WPDES permit with monitoring. This monitoring is necessary for implementation of ch. NR 207, Wis. Adm. Code, entitled "Water Quality Antidegradation." The recommended monitoring in Table 4 should be included in the WPDES permit along with, or in place of the recommendations in Tables 1 through 3 where the provisions of Table 4 are more restrictive.

Table 4 - Outfall -17

Mercury	2,3,7,8-TCDD
Acrolein	2,4,6-Trichlorophenol
3,3'-Dichlorobenzidine	Fluoranthene
Hexachlorobenzene	Pentachlorobenzene
1,2,4,5-Tetrachlorobenzene	Alpha-BHC
Beta-BHC	Gamma-BHC (Lindane)
Technical Grade BHC*	Chlordane
Dieldrin	4,4'DDT
Endosulfan	Endrin
Heptachlor	PCBs*
Toxaphene	

* - Technical grade BHC is not specifically a measured compound, it is considered to be the sum of the alpha-,beta-,gamma-, and delta-BHC isomers. In addition, the term "PCB" refers to each of the individual Aroclor mixtures with NR 105 criteria, namely Aroclors 1016, 1221, 1232, 1242, 1248, 1254, and 1260.

Monitoring is required at a minimum of once per year for the above-mentioned substances. That monitoring may be discontinued for any substance not detected during the first two years of the permit issuance period.

BIOMONITORING DISCUSSION/RECOMMENDATION

If there are any questions or comments, please contact either Bonnie Goodweiler at (608) 266-3484 regarding chemical-specific limitations, Bob Masnado at (608) 267-7662 regarding whole effluent toxicity evaluations, or either John Sullivan at (608) 267-9753 or myself at (608) 266-0156 regarding general issues.

a:\Niagaraa.bjg

Attachment

PREPARED BY:

APPROVED FOR SIGNATURE BY:

Bonnie Goodweiler
Water Resources Engineer

John R. Sullivan
Surface Water Standards Program Supervisor

James W. Schmidt, P.E.
Water Resources Engineer

cc:

Dennis Weisensel - LMD
Linda Vogen - LMD

Date: 6/18/1996
To: NOW File
From: Jim Amrhein  WT/2
Subj: Taste and odor in fish from Menominee River

I spoke today with Tom Thuemler (Fisheries Management - Marinette) regarding the recent history of taste and odor problems in the Menominee River. He said that in the past 2-3 years, complaints have gone down. They have not received any complaints since the process change at Champion.

Tom also indicated that the former fish manager on the Michigan side likewise felt the taste and odor problem had decreased.

I asked Tom whether or not there had been a change in fishing pressure. He stated that it is difficult to determine since regulations, which used to allow 5 walleyes per angler all year long, were cut back to allow only 1 walleye per angler before the fishing season. He said this action may have (negatively) impacted fishing pressure. Since there is no formal public boat access, access to the portion of the river below the NOW discharge is limited.

I also asked Tom about flow conditions because of the possibility that low flow could enhance taste and odor problems. Tom said the river flow was not unusually high the past two years - this year being an exception.

To summarize:

It looks as if taste and odor complaints have decreased. No unusual conditions have existed which may mask the taste and odor problem. While conducting a taste and odor test may not be appropriate at this time, I feel the Department should reserve the right to revisit this issue should a reasonable number of complaints be noted in the future.

Mark J. Akey
P.O. Box 147
Fox Lake, WI 53933

November 14, 1995

Jennifer Feyerherm,
Toxics Information Specialist
101 S. Webster ST.
Madison, WI 53707-7921

Dear Jennifer:

Thank you for sending me the 1991 - 1993 SARA 313 Data Summary Toxic Release Inventory. I thoroughly enjoyed reading the information that was available. I found that the format was easy to read and follow. The only thing that I thought could be added is on the charts with bar graphs, you might include numbers at the top or right of the bar to coincide with the number the bar represents i.e. 6.6 (million) or 749 (thousand). I think this would be useful as a quick reference guide.

Some of the following concerns I have are listed below.

The study that was listed in the 1993 SARA DSTRI on the Asthma in children (p. 47 & 48), hits close to home. The children in Port Edwards-Wood County, have been found to suffer from higher asthma rates than the state and national average. Port Edwards is located in the middle of a string of paper mills and factories in a North/South latitude. These people would be affected by air emissions from the north and south. The mills and factories would include: to the north; Biron Division of Consolidated Papers, Inc., Craft and Rapids Div. of CPI, and to the south; Port Edwards-Georgia Pacific, Nekoosa Papers, IBM, and Vulcan Chemicals.

On a more personal level, my 28 year old sister has been diagnosed with asthma for which she is undergoing treatment. We grew up on the east side of the Wisconsin River between the Biron Div. of CPI and the Craft/Rapids Div. of CPI. My father and his father had worked for the Biron Div. of CPI for 49 and 44 years respectively. My grandfather died from emphysema in 1965 and my father has been diagnosed with emphysema. My sister and I were adopted so we do not have a family medical history.

I am also happy to see that Wisconsin has adopted a more stricter code than the EPA. I hope that we continue to be leaders in environmental standards of the future.

Jennifer Feyerherm
November 14, 1995

Being that I grew up on the Wisconsin River in Wis. Rapids-Wood County, I was not surprised to see that the Wis. River was at the top of the list of most polluted rivers. What surprised me is the amount (370,142 pounds) of pollutants being released into this waterway. This amount is too high considering the rate of cubic-foot flow that the Wisconsin River averages. I feel that something should be done to decrease this amount dramatically.

While growing up during the 1960's and 1970's, I saw the effects of the chemical pollutants had on the fish. Tumors, deformed spines, heads, fins & tails were frequent among fish caught during this period. In the late 1970's and early 1980's, the river became less polluted from stricter laws. Slowly, some of the fish, most often the smaller fish, became palatable. What bothers me now are the sulfites being dumped into the river. In 1987, I took 2 catfish caught below the Centralia Dam on the Wis. River in the village of Port Edwards-Wood County, to be tested by the DNR because of the foul taste and odor of the bottom-feeding catfish. A fisheries manager, Steve ?, in the Wis. Rapids DNR office received the fish from my possession and transferred them to the State Lab for analysis.

The results were rather disappointing. The fish were tested for mercury and PCB's, which were well under safe edible guidelines. I had no concern over these pollutants. I was more concerned with what I believed were sulfites which was responsible for rendering catfish unpalatable. This problem has also occurred in the northern pike and walleye pike, the latter of which has been significantly enhanced by the 15 inch size limit on walleyes which is forcing fishermen and women to eat large, less palatable fish.

I believe that sulfites accumulate in fish due to the fact that larger fish have an off-flavored taste more so than smaller fish. I have also noticed that northern pike caught in the Wis. River tend to be softer when pickled than fish caught out of other bodies of water. I feel that this is an issue that should be addressed. If the pollutants are not dangerous, but are affecting the quality of the fish to the point that they become unedible, then something must be done to control the source point that is polluting the fish and the river.

I do not know if the chemical (or chemicals) are sulfites, but I do believe that whatever they are, they are coming from the bleaching process in the pulp industry. I believe with all my convictions, that these chemicals should be identified and eliminated from our environment. I am prepared to spend the rest of my life in pursuit of this goal.

In closing, please forward a copy of this letter of my concerns over environmental quality of the Wis. River fisheries to anyone who you may feel that this information may find useful. If you know of anyone that I might contact pertaining to these concerns, please let me know who they are and how to contact them. Also, could you please send me copies of the EPA chemicals fact sheet. I would appreciate this very much and am looking forward to hearing from you.

Respectfully submitted,

Mark J. Akey

Mark J. Akey