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Aromatic Hydrocarbon Pathology in Fish Following a Large Spill into the Nemadji River, Wisconsin, USA

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On June 30, 1992, a train accident resulted in a rail car releasing 114,000 L of a complex mixture of aromatic hydrocarbons into the Nemadji River, a tributary of Lake Superior near Superior, Wisconsin (Table 1). Although the majority of the spilled material evaporated, damage to aquatic life was extensive. Several thousand fishes were killed and an inestimable number were exposed to low concentrations (< 5 mg/L) of the chemical concentrate for several weeks (Allen 1993). Fishes that survived the spill were examined within 7 days of exposure to determine the extent of injury when compared to fishes collected from the reference site. The liver, spleen, gill, and head kidney were examined for histopathology. Blood was collected to determine the severity of liver damage reflected by the presence of the serum enzymes (aspartate aminotransferase, alanine aminotransferase, and δ-glutamyl transferase).

MATERIALS AND METHODS

Seven days after the spill, fishes that survived the exposure were collected from the Nemadji River approximately 25 km downstream from the spill site. The St. Louis River, a tributary of Lake Superior 16 km northwest of the Nemadji River, was chosen as the reference site. Black bullhead *(Ictalurus melas),* northern pike *(Esox lucius),* white sucker *(Catostomus commersoni),* and walleye *(Stizostedion vitreum)* were collected the same day from both rivers using electroshocking gear (pulse DC). As the fish were collected they were placed on ice. Two to four hours elapsed from the time the fish were collected until processed for blood and tissue samples.

Length and weight were recorded for each fish. Sex and the degree of sexual maturity were noted. The spleen, one gill arch, head kidney, and dorsal lobe of the liver (approximately 3 g) were excised and placed into buffered formalin (100 ml

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formalin and 900 ml distilled H_2O containing 4.0 g of H_2PO_4 and 6.5 g HPO_4) for histopathological analysis. The tissues were prepared according to standard histological procedures for sectioning in paraffin (5-7 pm), and stained with hematoxylin and eosin. Touch imprints of the head kidney for morphologic assessment were collected on a glass microscope slide prior to fixing the tissue in buffered formalin. The air-dried touch imprints of the head kidney were stained with Wright-Giemsa and examined for hematologic abnormalities of erythrocytes exhibiting micronuclei and nuclear irregularities. Hematologic morphology and cell composition of the head kidney for each fish species from the Nemadji River were compared with the same species from the St. Louis River.

Table 1. Aromatic hydrocarbons in the chemical concentrate released into the Nemadji River (Allen 1993).

Compound	[1]Ratio Released (%)
benzene	45.0
dicylopentadiene	13.0
cyclopentadiene	7.0
toluene	6.0
C-10 hydrocarbon mix	5.5
C-6 hydrocarbon mix	3.5
styrene	3.0
C-5 hydrocarbon mix	2.8
1-pentene	2.5
cyclopentene	1.7
isoprene	1.5
C-9 hydrocarbon mix	1.4
naphthalene	1.3
1,3 butadiene	1.1
indene	1.0
other hydrocarbons	3.7
Total	100

[1]Percentages are by weight.

Blood was colleted using a 3 cc syringe and a one inch 18 gauge needle. All fish except the white sucker were bled from the caudal hemal arch; white sucker were bled using heart puncture. Whole blood was dispensed into a 4.0 ml polystyrene centrifuge tube and allowed to coagulate at 270 C for one hour prior to centrifugation (1500 x g) for five minutes. The serum was separated from packed red blood cells and placed on ice 24 h until frozen at -80° C for plasma enzyme analyses. Plasma enzymes for each fish species collected from the Nemadji River were compared to the same species collected from the reference site.

Human and veterinary tests that screen for liver damage can identify changes in specific enzyme activities associated with liver function (Zimmerman et al. 1968;

Schmidt and Schmidt 1985). Tissue-specific enzymes such as aspartate aminotransferase (AST), alanine aminotransferase (ALT), and δ-glutamyl transferase (GGTP) are commonly used as indicators of liver toxicity and can be measured in teleosts (Gingerich 1982). AST, ALT, and GGTP were analyzed at 25°C using an IL Monarch 2000 (Instrumentation Laboratory Company, Lexington, MA). GGTP was measured at an increase in rate of absorbance of p-nitroaniline which is directly proportional to the quantity of GGTP present at 405 nm. AST and ALT were measured by the decrease in absorbance at 340 nm which is directly proportional to the oxidation of NADH to NAD. The enzyme activities were expressed in International Units/liter (IU/L = 1 µmol of substrate utilized per min at 25° C). The differences in enzyme activities between the Nemadji River and the reference site (St. Louis River) were tested using a one-tailed Student's *t* with $P < 0.05$ (Zar 1984).

RESULTS AND DISCUSSION

Histopathological analysis of gill tissue in fishes collected from the Nemadji River showed an increase in basal hyperplasia, fusion of lamellar epithelia, excess mucous production, and swollen lamellae (Photos 1-4). Fishes from the reference site did not exhibit gill abnormalities. Liver, spleen, and head kidney in fishes collected from the Nemadji River were not histopathologically different when compared with the same species from the reference site. None of the head kidney imprints from either site exhibited identifiable hematologic abnormalities. Fish of the same species collected from the Nemadji River were not hematologically different than their counterpart from the St. Louis River.

Considerably more information is available on the impacts of aromatic hydrocarbons in mammals than in fishes. Aromatic hydrocarbons such as benzene are considered toxic to the cells of mammals, but only at high dosages (100 mg/L) and for extended periods of time (Snyder and Kocsis 1975). Although the histology of the liver, head kidney, and spleen in fish collected from the Nemadji River revealed very little information concerning the impact of the spill, histology revealed gill damage in all fishes collected from the Nemadji River and that the extent of the damage was severe as indicated by lamellar fusion and basal hyperplasia. The chemical irritants may have caused the lamellar epitheliums to proliferate as reflected by an increase in number and size of epithelial cells between the spaces of the lamellae (Post 1983). Damage to gill tissue may result in long-term consequences, particularly if the tissue damage is not repairable. The end result would be reduced flow of oxygen-enriched water to lamellar tissues and ultimately a reduction in the fish's performance capacity (ie., ability to forage and escape predation).

Fishes collected from the Nemadji River when compared to the reference site experienced some degree of hepatoxicity 7 days after the chemical spill. Of the three serum enzymes, AST was significantly greater in the white sucker and black

Photo 1

Photo 2

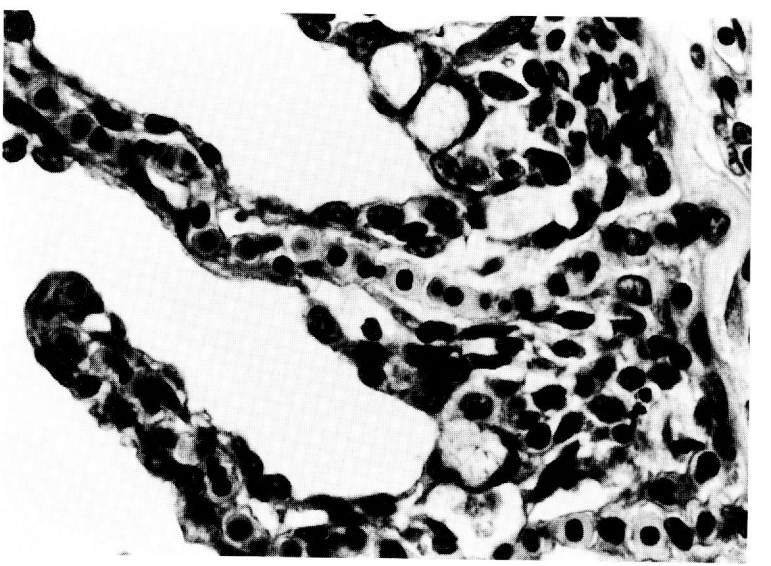

Photo 1. Histopathological analysis of gill tissue in northern pike collected from the Nemadji River showed swelling of lamellae cells and degenerate epitheliums (arrows). **Photo 2.** A normal gill epitheliums in northern pike collected from the reference site.

Photo 3

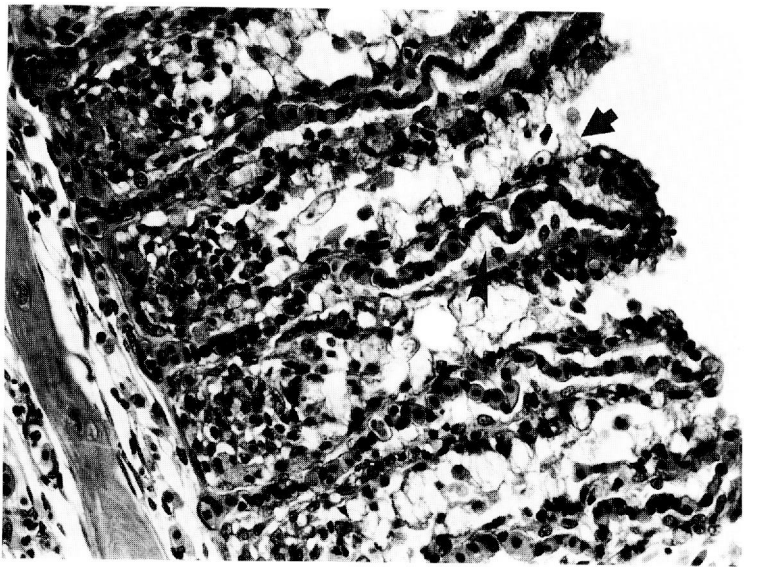

Photo **4**

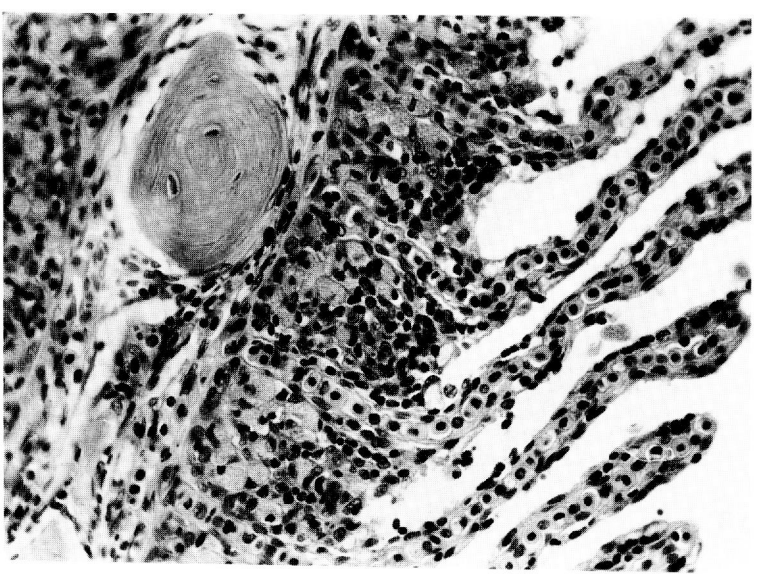

Photo 3. Gill tissue from white sucker collected from the Nemadji River having excess mucous (arrow) and lifting of the lamellae epitheliums (arrowheads). **Photo 4.** Gill tissue from a white sucker collected from the reference site showing normal gill epitheliums.

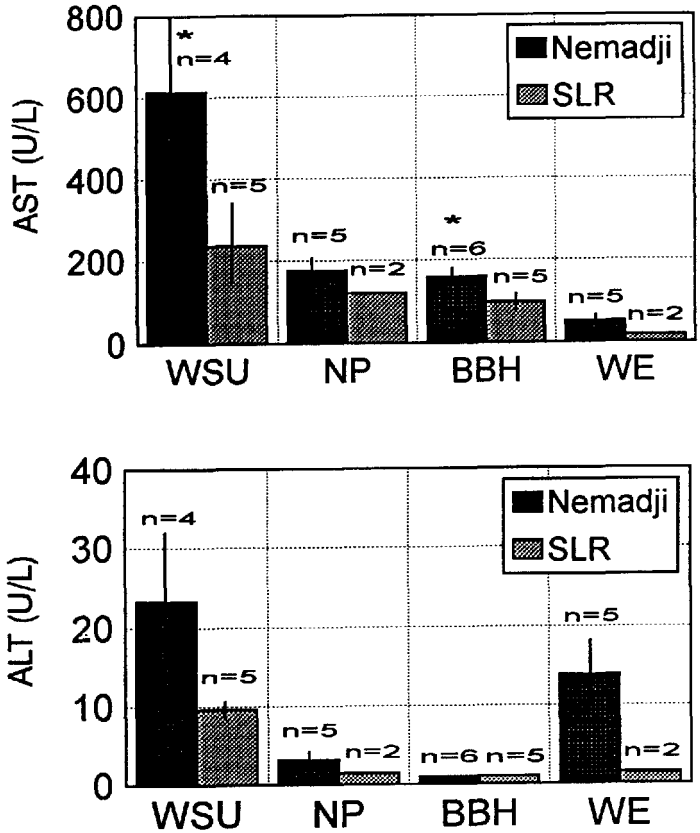

Figure 1. Mean liver enzyme activity (International Units/L) (± standard error) for aspartate aminotransferase (AST) and alanine aminotransferase (ALT) in white sucker (WSU), northern pike (NP), black bullhead (BBH), and walleye (WE) collected from the Nemadji River and the St. Louis River (SLR). *indicates significant difference from the reference site (P<0.05).

bullhead collected from the Nemadji River (P < 0.001) (Fig 1A). Although not significant, there were trends toward elevated levels of AST in northern pike and walleye collected from the Nemadji River. And although not significant, there were trends toward elevated levels of ALT in white sucker, northern pike, and walleye collected from the Nemadji River when compared with the same species collected from the St. Louis River (Fig lB). The serum enzyme GGTP was not detectable in all species from both rivers. Environmental pollutants have been demonstrated to affect aminotransferase activities in fish exposed to aromatic hydrocarbons (Gingerich and Dalich 1978; Dalich et al. 1982; Dange 1986), chlorinated hydrocarbons (Folmar et al. 1993; Rhodes et al. 1985), an herbicide (El-Deen and Rogers 1993), and heavy metals (Folmar et al. 1993; Hilmy et al.

1985; Rhodes et al. 1985). Gingerich and Dalich (1978) demonstrated a single dose of monochlorobenzene (1.0 ml/kg) elevated plasma ALT activity (56 IU/L) in rainbow trout *(Oncorhnychus mykiss)* for 72 hours which was subsequently followed by a decrease 24 hours later (26.8 IU/L). They confirmed the presence of degenerative changes in the liver as early as 8 hours after the treatment and evidence of only minor by damage 48 hours. Gaudet et al. (1975) reported plasma ALT levels in healthy rainbow trout were consistently 5 to 10 times lower than AST levels similar to that observed here for all four species of fishes analyzed from the reference site.

The growing concern of harmful effects and potential hazards of contaminants in the environment has been the motivating force to identify alternative tools for diagnostic use. Although, the application of serum enzymes as quantitative tools in field studies are impractical when the time of exposure to the time of the sample, the magnitude of the exposure (acute vs sublethal), and the variation that exists between individual fish as well as among fish groups are unknown. However, in conjunction with other indices of contaminant exposure, the relatively low cost of analyses and ease in collecting a small blood sample makes serum aminotransferase activity practical as a descriptive and qualitative indicator of exposure.

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REFERENCES

Allen PD (1993) Natural resource injury resulting from the June 30, 1992 Nemadji River spill. Natural Resource Damage Assessment Report, USFWS, Green Bay, WI

Dalich GM, Larson RE, Gingerich WH (1982) Acute and chronic toxicity studies with monochlorobenzene in rainbow trout. Aquat Toxicol 2:127-142

Dange AD (1986) Metabolic effects of naphthalene, toluene or phenol intoxication in the cichlid fish tilapia, *Oreochromis mossambicus:* changes in aminotransferase activities. Environ Pollut 42:311-323

El-Deen MAS, Rogers WA (1993) Changes in total protein and transaminase activities of grass carp exposed to diquat. J Aquat Anim Health 5:280-286

Gaudet M. Racicot JG, Leray C (1975) Blood and liver enzymes in rainbow trout *(Salmo gairdneri* Rich.) with emphasis on their diagnostic use: Study of CCl_4 toxicity and a case of *Aeromonas* infection. J Fish Biol 7:825-835

Gingerich WH, Dalich GM (1978) An evaluation of liver toxicity in rainbow trout following treatment with monochlorobenzene. Pro West Pharmacol Soc 21:475-480

Gingerich WH (1982) Hepatic toxicology of fishes. Aquatic toxicology, Raven Press, New York

Folmar LC, Bonomelli S, Moody T, Gibson J. (1993) The effect of short-term exposure to three chemicals on the blood chemistry of the pinfish *(Lagodon rhomboids),* Arch Environ Contam Toxicol 24:83-86

Himly AM, Shabana MB, Daabees AY (1985) Effects of cadmium toxicity upon the *in vivo* and *in vitro* activity of proteins and five enzymes in blood serum and tissue homogenates of *Mugil cephalus.* Comp Biochem Physiol 8lC:145-153

Post GW (1983) Textbook of Fish Health. TFH Publications, New Jersey

Snyder R, Kocsis JJ (1975) Current concepts of chronic benzene toxicity. CRC Crit Rev Toxicol 3:265-288

Rhodes L, Casillas E, McKnight B, Gronlund W, Myers M, Olson OP, McCain B (1985) Interactive effects of cadmium, polychlorinated biphenyls, and fuel oil on experimentally exposed english sole *(Paraphrys vetulus).* Can J Fish Aquat Sci 42:1870-1880

Schmidt E, Schmidt FW (1985) Aminotransferases in human pathology and clinical chemistry. In: Christen, Metzler DE (eds) Transaminases, John Wiley and Sons, New York, p 624

Zar JH (1984) Biostatistical Analysis. Prentice-Hall, Inc, Englewood Cliffs, New Jersey

Zimmerman HJ, Dujovne CA, Levy R (1968) The correlation of serum levels of two transaminases with tissue levels in six vertebrate species. Comp Biochem Physiol 25:1081-1089