

**USE OF THE NITROBLUE TETRAZOLIUM (NBT) ASSAY TO  
DETERMINE THE EFFECTS OF CONTAMINANTS ON  
BROWN BULLEHAD**

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**Introduction**

Immunological biomarkers can play an important role in monitoring the health of a population as well as indicating potential harm from environmental contaminants (Weeks *et al.*, 1992). One method of monitoring the non-specific response is through the metabolic activity of neutrophils using a nitroblue tetrazolium (NBT) test. This assay focuses on the ability to produce oxygen radicals ( $O_2^-$  and  $OH^-$ ) and subsequently reduce a soluble nitroblue tetrazolium dye to insoluble formazan (Anderson *et al.*, 1992).

Studies of the Black River, near Lorain, Ohio, have linked elevated polynuclear aromatic hydrocarbons (PAH) in the sediment with a high frequency of tumors in the local brown bullhead (*Ameiurus nebulosus*) population (Baumann *et al.*, 1990). Contradictory studies have shown enhancement (Rice and Weeks, 1989; Robohm, 1986) and suppression (Faisal and Huggett, 1993; Weeks *et al.*, 1990) of the immune response due to chronic exposure to various environmental contaminants including PAH's. Fish from a PAH contaminated site were tested to determine if they would show a difference in neutrophil activity compared to those from a reference site.

**Methods**

A drop of blood from a sample was placed on each of two coverslips and incubated in humidified chambers at room temperature (22°C) for 30 min. During this time, the neutrophils adhered to the glass. Each coverslip was thoroughly rinsed with 0.85% saline solution and inverted onto a slide with drops of 0.2% NBT solution. After another 30 min incubation period, slides were examined under a photo-microscope. Four random fields of positive, dark blue staining cells were observed for each coverslip. A picture was taken of each field (280x) for later counting of neutrophils. Activated neutrophils for each field were added together to give a total number of cells per slide.

**Results**

In May, 1993, mean neutrophil counts in brown bullhead from the Black River were more than five times higher than those from Old Woman Creek. Fish collected from the Black River in September 1993, were found to have a mean over five and one half times higher than Old Woman Creek bullhead. Comparisons between sites from the same collection period found neutrophil activity to be significantly higher using the Student's *t* test ( $p \leq 0.05$ ). The results indicate that the non-specific immune response may have been stimulated by the presence of PAH compounds or by the resulting pathology.

Table I. Mean counts of activated neutrophils for brown bullhead collected from the Black River and Old Woman Creek (OWC) in May and September, 1993. Black River fish were found to have significantly higher activation using the Student's *t* test.

Site	May 1993			Sept 1993		
	n	x	SE	n	x	SE
Black River	21	160.2	43.8	22	101.7	13.4
OWC	12	30.2	9.82	19	17.8	1.44

## Discussion

The immune response is a complex system that can be altered by a variety of factors. The data shows neutrophil activation was higher in May than September. Reproductive conditions may have affected the response. At the time of the May collection, bullhead were actively spawning. However, water temperatures and other environmental factors may have played a role as well.

The NBT test appears to have potential as an effective field assay. It is quick, uncomplicated, and inexpensive. In our trials, the assay produced significantly higher results at the contaminated location despite considerable seasonal variation within sites. This assay cannot pinpoint a specific agent as the cause of altered neutrophil activity unless used with a suite of other tests (e.g. phagocytic indices, Mixed Function Oxidase induction). However, it shows promise as an early indicator of a population's health. More studies with different species and contaminants must be done to ultimately determine its effectiveness.

## References

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