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Pulmonary Histopathology in Ring-Billed Gulls (*Larus delawarensis*) from Colonies near Steel Mills and in Rural Areas

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Combustion of fossil fuels releases complex mixtures of gaseous and particulate contaminants into the environment. There is an ongoing debate over the health-related effects of exposure to airborne contaminants in urban environments. Some effects of exposure to high levels of air pollution can include: lung tissue damage, inflammatory responses, impairment of pulmonary host defenses, acute changes in lung function and respiratory symptoms, chronic changes in respiratory epithelium, and significant cumulative lung burdens after chronic exposure that can disrupt lung function and produce long-term pathologic effects (reviewed in Folinsbee, 1992). Numerous studies have investigated the morbidity and mortality associated with exposure to contaminants in areas prone to air pollution episodes. For example, in humans living in areas of high industrialization and urbanization, the incidence of hospital admissions for respiratory-related conditions, and the incidence of deaths from cardio-pulmonary complications is correlated with exposure to airborne contaminants (e.g., Dockery and Pope, 1994; Bates et al., 1995; Pope, 1999). Additionally, associations in humans between the incidence of asthma, pneumonia and airway hyperreactivity and episodes of high particulate pollution have been documented (Pope and Dockery, 1992; Delfino et al., 1994). These studies demonstrate that increased mortality in the general population is observed at times when concentrations of ambient air pollutants are high. Despite our increased knowledge of the health consequences of exposure to air pollution, few studies have investigated the direct pathologic effects of exposure to high levels of air pollution in environmental and occupational settings in animals and humans. One study examining the biochemical and morphological alterations in the lungs of mice exposed to air pollution in a traffic tunnel showed significant pulmonary histopathological effects associated with chronic exposure (6 months; Wang et al., 1992). These changes included anthracosis or pneumoconiosis, chronic inflammation and emphysema. In humans, inhalation of coal dust caused anthracosis, concomitant catarrhal-desquamative bronchitis, lymphoid tissue hyperplasia, emphysema, along with granuloma formation (Ivanova, 1990). Previous studies in humans have shown increased respiratory morbidity correlated with exposure to particulates with an aerodynamic diameter $< 10\mu m$ (PM_{10}) in cities with steel operations (e.g., Schwartz, 1994).

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In this study, we examine histopathologic changes in lung tissue of adult ring-billed gulls (*Larus delawarensis*), colonial-nesting sea birds of the Great Lakes. Gulls were examined from two colonies situated in cities with large integrated steel operations and from two rural locations, remote from cities and point sources of air pollutants. We have previously investigated colonies of herring gulls (*Larus argentatus*) inhabiting these four locations and showed that rates of germline minisatellite mutations (tandemly-repeated non-coding DNA sequences) were statistically significantly higher in colonies near steel mills compared to rural locations (Yauk and Quinn, 1996; Yauk et al., 2000). A significant negative correlation was found; mutation rates increased with decreasing distance between nests and steel mills (Yauk et al., 2000). We hypothesized that the increased mutation rates resulted from exposure to industrial chemicals through two possible routes: (a) ingestion of contaminated food and (b) inhalation of air contaminants. Hundreds of chemicals and high levels of air particulates are released into the environment through steel processing. In this study we use ring-billed gulls as a surrogate for herring gulls because they are much more abundant in the Great Lakes than the herring gull (nesting in the tens of thousands versus hundreds at typical herring gull colonies). It has been suggested that adult birds may be sensitive monitors of environmental contamination by gases and airborne particulates at low concentrations. The large amounts of oxygen required for flight coupled with a high metabolic rate suggest the potential application of birds as sentinel species for airborne chemical exposures (Brown et al., 1997).

MATERIALS AND METHODS

Our two rural locations were Presqu'ile Provincial Park (44° 00' N, 77° 43' W), in Lake Ontario, Canada and Chantry Island in Lake Huron (44° 29' N, 81° 23' W), Canada. These colonies are removed from point sources of pollutants such as industries and highways. Our two contaminated colonies were situated in large cities, next to large integrated steel operations. These colonies are Hamilton Harbour (43° 15' N, 79° 51' W) in Ontario, Canada, on Lake Ontario, and East Chicago on Lake Michigan in Indiana U.S.A. (41°64' N, 87°45' W). The ring-billed gulls sampled in Hamilton nested less than two kilometers from the industrial coking ovens. The gulls in East Chicago nest on the industrial grounds of LTV Steel, the highest concentration of steel industries on the Great Lakes.

Adult ring-billed gulls were captured with drop traps set over their nests late in the incubation season. We captured 25 adults (approximately equal sex ratio) from each of the four locations. The gulls were sacrificed by decapitation with a guillotine and immediately dissected. Tissues fixed overnight in buffered formalin at room temperature were later embedded in paraffin wax. Serial sections (5 μm) were cut and stained with hematoxylin and eosin and examined by light microscopy. One cross-section from each lung was evaluated in which primary,

secondary and tertiary airways, as well as the respiratory epithelium (the alveoli) were represented. Histological assessments were conducted by one pathologist, who was unaware of the treatment group or origin of the individual animals. During the initial examination, the pathologist noted general tissue condition, the types and prevalence of pathological and artifactual changes (McLaughlin et al., 2000). For the second assessment, the lungs were examined in detail and numerical scores were assigned to represent the presence and/or severity of changes in the different structures comprising the pulmonary parenchyma. The rankings were as follows: 0=no reaction; 1=mild reaction; 2=moderate reaction; 3 =severe reaction. The five histopathologic changes measured in each of the ring-billed gulls were: (1) anthracosis - a measure of particles of carbon trapped in the lungs, commonly encountered in areas with heavy industrialization, high auto emissions or dusty locations, (2) periarteritis - an accumulation of mononuclear and other inflammatory cells in the space adjacent to the pulmonary arterioles, (3) bronchitis - heterophil accumulation in the lamina propria and/or bronchial lumen resulting from bronchial irritation, (4) granuloma formation – a localized, chronic inflammatory response mounted in birds which is characterized by multinucleated giant cells around necrotic foci, and (5) pleuritis – inflammation of the external surface of the lung which is intimately associated with the adjacent air sacs. Our analysis also included cases in which the presence of both granulomas plus periarteritis were seen, as well as an analysis of the total median score derived from the sum of pathological changes from birds from each of the four sites. The pathologic results were analyzed using a Kruskal-Wallis one way analysis of variance to test the null hypothesis that there is no difference in population medians of pulmonary pathologic changes (Daniel, 1978).

RESULTS AND DISCUSSION

The lung tissue samples from the four study locations were well preserved and there were minimal artifacts due to handling. There were no significant differences in any of the responses among birds from the four locations (Table 1). Chantry Island, a rural site, had the highest pathology rankings in all categories except granulomatous pneumonia, but these differences were not statistically significant. Surprisingly, East Chicago, presumed to be the most contaminated location, showed the lowest prevalence of severity of histopathology for each of the pathological changes assessed, although this difference was not statistically significant ($p=0.139$). The East Chicago site is associated with high levels of airborne polycyclic aromatic compounds (PACs) as well as polychlorinated biphenyls (Simcik et al., 1997). The site is situated near numerous interstate highways, large volumes of automobile traffic associated with the metropolitan area of approximately 8 million people, and a high concentration of industry. Therefore, it seems unlikely that the gulls in East Chicago were exposed to lower levels of contaminants than the rural sites. Hamilton, our second steel site, is the

site of the greatest concentration of steel industry in Canada. Levels of PACs associated with inhalable air particulate (PM_{10}) collected downwind of the steel industries are known to be elevated relative to air samples collected further from the sources (Legzdins et al., 1993). Therefore, it is unlikely that exposure to anthropogenic airborne contaminants were similar between the industrial and rural sites sampled.

Table 1. Histopathological changes in the lungs of ring-billed .

	RANK					
Pathologic Response:	HH*	LTV*	CI**	PP**	Chi-Square df = 3	p-value
Anthracosis	53.7	42.3	53.7	52.0	3.04	0.386
Granulomas	57.6	42.1	53.1	49.2	4.39	0.222
Periarteritis	49.8	44.7	56.7	50.7	2.49	0.477
Pleuritis	51.0	45.0	53.0	53.0	2.40	0.493
Bronchitis	51.1	46.0	52.9	52.0	1.06	0.786
Granulomas and arteritis	55.0	40.6	56.9	49.4	5.06	0.168
All lesions Combined	54.6	39.8	57.5	50.2	5.49	0.139

The sum of ranks describes a combination of the intensity and frequency of the different pathological changes seen in the sample of birds from each colony.
* industrial sites, HH= Hamilton Harbour; LTV=East Chicago
** rural sites, CI=Chantry Island; PP=Presqu'ile Provincial Park

We suggest several hypotheses to explain our data: (a) gulls from the different colonies are exposed to pathogens and/or contaminants during their life span that induce similar effects, (b) the period of time spent at the colony is not long enough to result in differences in lung pathology, (c) the lungs of ring-billed gulls are not sensitive targets for measuring the potentially damaging effects of exposure to air pollutant, (d) the histologic endpoints measured are not relevant to exposure to air pollutants from steel industries, (e) acclimation occurs in ring-billed gulls from the industrial locations.

Ring-billed gulls are opportunistic foragers and their diets may include fish, aquatic insects, worms, vegetation and garbage (Brown and Ewins, 1996). Data on the diet of the ring-billed gulls over the sampling period were not collected. Therefore, it is not possible to evaluate potential exposure to pathogens in the foraging area. Curiously, anthracosis, or dark, inorganic granules within alveolar macrophages and bronchial epithelium likely resulting from the inhalation of carbon compounds, was similar between the sites. This measure is rarely of any

clinical significance, but we predicted that this measure would be the most informative in demonstrating exposure near the steel industries. The gulls may forage many kilometers from the colony location although they return regularly during the breeding season to mate, incubate eggs, feed chicks and roost at the colony. Pathogen exposure as a result of feeding in dumps or farmers' fields could result in similar expression of some of the endpoints measured, but, it is interesting that anthracosis was not different among the colonies. However, anthracosis in these ring-billed gulls may not reflect short term exposure to particulates from local point sources. Rather, the gulls' life history and age are important factors in evaluating the significance of this particular endpoint. If the rural gulls sampled are wintering in areas near sources of combustion-related particulates, these gulls would be expected to accumulate anthracosis over this period. The gulls arrive at the breeding colony in the month of April, and remain near the colony during the breeding season. Sampling in June provides a period of exposure to surrounding airborne contaminants over two months. The gulls then disperse from the colony in August or September to congregate along the lower Great Lakes before southward migration (Ryder, 1993). The Great Lakes ring-billed gulls do not migrate to any distinct wintering area. Rather, the gulls may winter in the south eastern United States or even stay in open water within the Great Lakes (Ryder, 1993). Therefore, unfortunately, the wintering locations cannot be predicted. Furthermore, the ages of the birds sampled are unknown. The gulls sampled had adult plumage, indicating that they were a minimum of three years of age (Ryder, 1993). Therefore, a further possibility is that the period over which the ring-billed gulls are challenged with industry-specific pollutants is not long enough each year, or lifetime exposure is too variable within the population, to develop pollutant-associated pathology that is statistically detectable. Future studies should investigate the histopathology of young of the year at these colonies. An exact period of exposure could be quantified for these gulls, and histopathology would result exclusively from their immediate environment on the colony.

From this study, it appears that histopathologic changes in lung cells of ring-billed gulls may not be appropriate targets as biomarkers for exposure to air pollution possibly because exposure for two to three months does not result in lung pathology. Very little is known about the pathophysiology of particle exposure in bird lungs. The mechanisms responsible for the clearance of particulates from the bird's respiratory system following prolonged dust exposure are poorly understood (Brown et al., 1997). Therefore, it is possible that the specific histopathologic endpoints observed in the gulls do not respond in the expected manner to air pollution.

Finally, the most interesting hypothesis put forth to explain the lack of difference in histopathologic endpoints among the ring-billed gull colonies is that

of acclimation. Acclimation is a phenotypic response to an environmental challenge that may enhance performance allowing organisms to persist under conditions that might otherwise be lethal (Hoffman, 1995). This process occurs in many organisms. For example, plants develop mechanisms of endurance that allow them to withstand stressors such as UV-B radiation, chilling and freezing, salinity and flooding, as well as acclimation to anthropogenic contaminants like herbicides and heavy metals (reviewed in Prasad and Rengel, 1998). Other examples include increased survival in fish resulting from acclimation during pre-exposure periods to heavy metals like cadmium (Hollis et al., 1999) or zinc (Alsop et al., 1999). It is possible that prolonged exposure to airborne contaminants at the steel sites results in the development of better clearance mechanisms in the ring-billed gulls at that site. This could explain similar levels of severity of anthracosis measured among the steel sites and rural sites. The gulls have a minimum exposure of 2 months, however, wintering grounds and age of the gulls is unknown and should be more thoroughly investigated should this hypothesis be pursued.

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