The Big Picture

Mapping SARS in Hong Kong

Epidemiologists have long used maps to track the spread of disease, and in the past decade, geographic information system (GIS) technology has added powerful new tools that help reveal far more than simply the "where" and "when" of epidemics. Now P.C. Lai of the University of Hong Kong and colleagues show how GIS technology can be used during an acute infectious disease outbreak to reveal crucial real-time, quantitative information, such as the direction of superspreading events (in which one person infects more than the typical three or fewer others) and distinct disease hot spots [*EHP* **112:1550–1556**]. Reaching beyond typical descriptive mapping, this study demonstrates the rich depth of GIS capabilities in analyzing patterns of disease spread from various perspectives.

Bird's-eye view of SARS. Using GIS technology, researchers have mapped how SARS spread in Hong Kong to help predict patterns of future infectious disease epidemics.

The global outbreak of severe acute respiratory syndrome (SARS) in late 2002 and into 2003 ultimately accounted for more than 8,000 cases in 29 countries, according to the World Health Organization. About 20% of the cases were in Hong Kong. Lai and colleagues applied geostatistical methods to analyze the spread of SARS in Hong Kong during this time period.

The investigators analyzed an integrated database that contained clinical and personal details on the 1,755 Hong Kong patients confirmed to have had SARS. They plotted patient residence addresses using a GIS to research such aspects as the superspreading event responsible for more than 300 cases in the Amoy Gardens housing development and microclusters of SARS cases (where the density of infection varied widely between districts).

The geostatistical analysis was conducted at three levels: elementary (visual inspection of geographical phenomena), cluster analysis to identify hot spots, and contextual analysis to explain relationships between geographical phenomena. Among the methods the researchers applied were nearest neighbor analysis, which discerns nonrandom distribution of cases and is often used by scientists studying species distribution. For another analysis, they used the kernel mathematical method to create a series of statistical "surfaces" to reveal daily changes in disease hot spots.

Elementary analysis revealed the spread of the disease: a clear clustering of cases in certain districts of the Kowloon peninsula, where Amoy Gardens is located, and in Hong Kong's New Territories region. Next, cluster analysis produced a series of 12 kernel maps based on date of symptom onset. These maps showed the density of SARS patients (adjusted for underlying population density) on typical days representing different stages of the 16-week outbreak; this demonstrated the development and dissipation of disease hot spots over the course of events. Another sophisticated analysis produced a map that summarized SARS hot spots by infection rate per 1,000 population, indicating that the urban population was at the highest risk.

With contextual analysis, the researchers developed origin-and-destination plots for three superspreading event clusters: Prince of Wales Hospital, Amoy Gardens, and Lower Ngau Tau Kok Housing Estate. The Prince of Wales Hospital cluster showed a northwest–southwest trend of disease spread that extended over most of Hong Kong (visitors to SARS patients at Prince of Wales Hospital spread the disease as they returned home, the authors observed). The Amoy Gardens cluster was comparatively more localized, while the Lower Ngau Tau Kok cluster was the most contained of the three.

The authors cautioned of limitations in applying GIS technology to infectious disease epidemiology and outbreak investigation, among them the occasional lack and unavailability of the necessary data. Still, the authors wrote, "integration of GIS technology into routine field epidemiologic surveillance can offer a scientifically rigorous and quantitative method for identification of unusual disease patterns in real time." When linked with point-of-care databases and other sources of environmental data (including meteorological, transportation, and topographical information), such geospatial intelligence has the potential to rapidly recognize, locate, and monitor disease outbreaks. **–Laura Alderson**

A Hazard *in Utero*?

Bisphenol A More Potent than Expected

Environmental estrogens are a structurally diverse group of chemicals that partially mimic the effects of endogenous estrogens. Scientists believe the wide use of environmental estrogens such as bisphenol A (BPA), a component of epoxy resins and polycarbonate plastics, may help explain the rising incidence of birth defects and certain cancers. It is further believed that the developing embryo is more vulnerable to the effects of environmental estrogens than adult animals, but until now it has been difficult to determine these effects directly in embryos. In this issue, Josephine G. Lemmen of the Netherlands Institute for Developmental Biology and colleagues investigate the use of a new transgenic mouse model to study such effects [*EHP* **112:1544–1549**].

During the late 1990s, it was first suggested that prenatal exposure to BPA might cause reproductive abnormalities; experimental data, however, led to contradictory findings. For instance, one 1999 study showed prostate enlargement in offspring of BPA-exposed mice, but other studies reported no effect. Although BPA concentrations in amniotic fluid at 15–18 weeks' gestation have been shown to be five times those in the serum of pregnant and nonpregnant women—suggesting possible accumulation in the embryo—this could not be confirmed through animal experiments.

Lemmen and her colleagues recently developed a transgenic mouse in which estrogen-responsive elements are coupled with the reporter enzyme luciferase. Direct activation of estrogenic receptors (ERs) is detected photometrically by measuring luciferase activity, which allows quantitative and time-course analysis of target gene activation *in vivo*. The C57Bl/6J mouse strain used in this model had previously been shown to be especially sensitive to the effects of estrogen exposure.

This model was used to evaluate the ability of BPA to activate endogenous ERs in mouse embryos, as compared with the strong

Flat Earth

estrogens diethylstilbestrol (DES) and 17β-estradiol dipropionate (EP). Exposure of pregnant mice to varying dosages of all three estrogens activated the endogenous ERs in their embryos. Exposure to DES and EP showed a dose- and time-dependent induction of luciferase activity. For all DES exposures, peak activity was seen at 8 hours after exposure. For EP, peak activity was seen at 24 hours after exposure.

Like DES, BPA showed a transient induction of luciferase activity. Surprisingly, though, BPA was found to be more potent *in vivo* than would have been expected on the basis of its activity *in vitro*. *In utero* luciferase activation by BPA in transgenic embryos at 8 hours after exposure was significant at a dosage as low as 1 milligram BPA per kilogram body weight, compared with controls.

One possible explanation for a higher potency *in utero* may be that *in vivo* BPA is converted to metabolites with enhanced estrogenicity, as some previous studies have suggested. Yet another explanation could be that BPA has a lower affinity with steroid-binding proteins present in serum, which gives it a greater bioavailability than, say, EP. However, these explanations cannot account for the *in vivo* versus *in vitro* potency of BPA as compared with DES, because neither has a high affinity to binding proteins.

Although BPA's intrinsic activity is lower than that of DES or EP, it still was more potent *in vivo* than would be estimated from *in vitro* assays. Effects on individual embryonic organs have not yet been evaluated and could possibly provide even more sensitive end points than whole embryos. Although the Lemmen study model showed that the effects of BPA did not persist like those of the other estrogens, its biological effects in exposed embryos should continue to be assessed, perhaps with other types of models. **–Julian Josephson**

The Safety of Xenoestrogens

Challenging the Genomic Model of Effects

Current thinking holds that environmental estrogens cause endocrine disruption when these steroid mimics enter the cell's nucleus and turn genes on or off, or up or down, by binding to DNA. According to this genomic–nuclear pathway model, many xenoestrogens are viewed as harmless to humans and wildlife because exposure to high levels of chemical is necessary before there is a change in gene expression. However, the genomic model assumes a long, multistep process of macromolecular synthesis; it does not fully account for empirical evidence that the signal response to some hormones is so fast it must be initiated outside the cell via membrane receptors connected to fast-acting molecules. An alternative membrane-initiated hypothesis is just beginning to be addressed and tested. This month, Nataliya N. Bulayeva and Cheryl S. Watson of the University of Texas Medical Branch report experimental evidence that challenges the prevailing genomic paradigm of endocrine disruption [***EHP* 112:1481–1487**].

For their experiments, the scientists employed a prolactinoma cell line derived from a rat pituitary gland that has been a model experimental cell line for over 30 years. A subline of these cells that exhibits fast responses and is sensitive to estrogens at small doses provides a good test system for the study of nongenomic responses to estrogenic compounds. This system allows researchers to investigate questions about mechanisms by measuring functional end points of estrogen action, such as prolactin secretion, which increases upon exposure to estrogen.

Extracellular signal–regulated protein kinases, or ERKs, belong to a large class of enzymes involved in cell signaling pathways that generate signals to multiple end points. They are good indicators of nongenomic estrogenic activity because ERK activation is mediated by phosphorylation, a signal from outside the cell. When ERKs are activated by exposure to estrogens or compounds that mimic estrogens, the cell's medium turns yellow. The yellow product, which correlates with the amount of phosphorylated ERK identified by an antibody, can be measured so precisely that small changes in levels of phosphorylated ERK can be detected.

Bulayeva and Watson compared ERK activation by the most potent endogenous estrogen—estradiol—with activation by three major classes of xenoestrogens: organochlorine pesticides (endosulfan, dieldrin, and DDE, a DDT metabolite), detergents used in plastics manufacturing (*p*-nonylphenol and bisphenol A), and coumestrol (a phytoestrogen present in alfalfa sprouts, soybeans, and sunflower seeds/oil). The responses were measured at different concentrations over a 3- to 30-minute time course. Affected points along the activation pathway were subsequently investigated by the addition of specific inhibitors to each pathway participant.

The results showed that every xenoestrogen tested, except bisphenol A, exhibited strong ERK activation. Unexpectedly, individual compounds produced the effect at different times and concentrations specific to the particular compounds. Also, individual compounds were found to trigger specific pathways within the nongenomic signaling network leading to different end points. Coumestrol, endosulfan, and *p*-nonylphenol had an effect at extremely low picomolar levels, as low as estradiol. The authors concluded, "These very low effective doses for xenoestrogens demonstrate that many environmental contamination levels previously thought to be subtoxic may very well exert significant signal- and endocrine-disruptive effects, discernable only when the appropriate mechanism is assayed."

This study sheds new light on the conundrum of why exposure to concentrations of environmental estrogens deemed safe by the genomic model exhibit well-documented harmful effects on wildlife. It raises new concerns about the effect of xenoestrogens on human health and important questions about the adequacy of current environmental protection policy and regulations based on the genomic model. **–Mary Eubanks**

New model explains differences. Researchers compared ERK activation by three classes of environmental estrogens found in pesticides, plastics, and plants to that of estradiol. Results showed that, unlike previously believed, not all xenoestrogens act the same.

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