Epidemiological Approaches to the Effects of Endocrine Disrupting Chemicals on Human Health

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ABSTRACT
We reviewed recent reports on endocrine disrupting chemicals (EDCs) and have outlined various problems inherent in them. The specific behavioral objectives of this review were to understand the findings in the reports and to criticize and evaluate them in an academically appropriate manner. Since most of the human studies adopted epidemiological methods, it seems indispensable for those interested in EDC problems to understand this discipline. In accordance with the categories of epidemiological studies, we reviewed the so-called Skakkebaek report (descriptive epidemiology), dioxins discharged by incineration facilities and neonatal mortality rate (ecological study), dioxins in breast milk and thyroxine in infants (cross-sectional study), dioxins and breast cancer (case-control study) and the Seveso report (cohort study). We discuss several issues underlying the epidemiological problems found in studies on EDCs, for example, difficulty in risk assessment on the individual level and the presence of bias. To address these issues, we emphasize the usefulness of surveys of accidental or industrial-exposure cases. In our concluding remark, we stress the large contribution of epidemiologists to public health and the need for debates with opponents because they share our interest in the effects of EDCs.

Ever since the publication of “Our Stolen Future” by Colborn et al. (6), the question of whether our fertility, intelligence, and survival are being threatened by endocrine disrupting chemicals (EDCs) has attracted increasing interest. A number of papers concerning the effects of EDCs on humans, in addition to their effects on wild animals, have been published all over the world, in regard to causal relationships between EDCs and human health disorders. However, no final conclusions have been drawn. Such conclusions should await scientific evaluation of these papers from various aspects. Since most of the human studies have employed epidemiological methods, an understanding this discipline seems indispensable to a critical evaluation of these studies.

We, first review recent reports on EDCs and then point out various problems inherent in them. The specific behavioral objectives (SBOs) for readers are to correctly understand the findings, then criticize and evaluate them in an epidemiologically appropriate manner. In addition, those who wish to investigate the human health effects of EDCs need to establish working hypothesis and test it by using epidemiologic approaches.

BASIC KNOWLEDGE REGARDING EDCs
EDC List
About 12 million man-made chemicals have been registered by the Chemical Abstract Service
Effects of EDCs on Human Health

The US EPA has summarized the types of human effects of EDCs as follows (7):
1. Female reproductive and development effects
   1) Disruption of female reproductive function (normal sexual differentiation, ovarian function, implantation, and pregnancy)
2) Endometriosis
3) Breast cancer
4) Vaginal cancer
2. Male reproductive system effects
   1) Reduced sperm production capability and reproductive tract abnormalities
2) Testicular cancer
3) Prostate cancer
3. Hypothalamus and pituitary
   1) Alteration of neuroendocrine function both during development and in the sexually mature organism
4. Thyroid effects
   1) Adverse effects on normal growth and development

Reports on EDCs by Massmedia in Japan

We mention several topics that have appeared in recent published newspapers or journals in Japan as well as inquiries that we have received regarding the implications of epidemiological studies on EDCs.
1) An ecological correlation between neonatal mortality rates and the amounts of discharge of industrial wastes for incineration in Tokorozawa City, Saitama Prefecture was reported, and our comments were requested.
2) A newspaper reported complaints of headache and nasal discharge by residents living near a garbage collection facility in Suginami, Tokyo, and the Tokyo Metropolitan Government Office promised to investigate a causal relationship with chemicals, including EDCs, in general waste.
3) "Is juvenile delinquency related to EDCs?" A newspaper claimed a need for epidemiological studies, especially in relation to EDCs in the diet.
4) A weekly journal requested our comments on the high incidence of malignant neoplasms among residents living near a garbage incineration plant in Shintone Town, Ibaraki Prefecture.
5) "Any effect of dioxin-contaminated breast milk on infants?" A newspaper reported that the current level of dioxin contamination is twice the level 20 years ago.

The above are a few examples that appeared in newspapers or journals in Japan. An epidemiological approach seems to be essential to address the problem of whether EDCs are etiologically involved in the occurrence of these disorders.

Epidemiological Methods of EDC Studies on Human Health

An EPA report (7) stated that relevant and adequate epidemiological studies and case reports are preferable. Several alternatives are suggested in the absence of such information, including pertinent experimental animal toxicology studies and in vitro studies to elucidate the mechanisms of toxicity. In particular, we are focusing on epidemiological methods to evaluate the human health effects of EDCs.

BASIC KNOWLEDGE OF EPIDEMIOLOGY

Definition and Characteristics

Epidemiology may be defined as a study of the distribution and determinants of health-related states or events in specified populations, and its application to the control of health problems (10). As epidemiologists we are primarily concerned with the distribution and frequency of diseases, but our main concern may be focused on a factor or factors that are related to the occurrence of diseases. Epidemiology is also a basic science that uncovers these factors. When we happen to discover a factor that is causally related to the occurrence of a disease, we can control the disease by taking advantage of the epidemiological discovery. In this sense, epidemiology may be characterized as an applied
science that generates clues to disease prevention.

To demonstrate the merits of epidemiology, we will quote an episode in the controversial problem of whether silicone breast implants are linked to immunologic sequelae, such as connective tissue disorders.

According to a report by Kondo (9), millions of women have undergone implantation of breast prostheses for cosmetic reasons. Over time, case reports of immunological disorders in women with silicone implants began to appear, and accumulation of such reports caused the public to suspect a risk of an autoimmune-like syndrome. At the same time, these reports troubled women with silicone prostheses who experienced the same disorders. Some of the women were reported to have sued silicone manufacturers, and a profusion of literature on this matter led epidemiologists to conduct case-control studies. The results showed consistent findings indicating no increased risk of developing immunologic disorders (18).

Taubes (17), however, pointed out the limitations of epidemiology in searching for subtle links between various factors and diseases.

Types of Epidemiological Studies

The Japan Epidemiological Association (JEA) (16) classified epidemiological studies into two major categories: observational and interventional (Table 1). In an observational study, disease occurrence can only be observed among already characterized people, those exposed or unexposed to EDCs, for example. There are two sub-categories of this category: descriptive and analytical epidemiology. In an interventional study, participants can be randomly allocated into groups exposed and unexposed to EDCs. This type of study is apparently the ultimate protocol for testing hypotheses, but careful consideration must be given to their ethical aspects.

Descriptive epidemiology literally describes the distribution of disease frequency in terms of person, place, and time. Its purpose is to formulate a hypothesis to identify its determinants. Analytical epidemiology is a method for investigating associations between diseases and possible determinant(s). The aim of such studies is to test hypotheses.

As shown in Table 1, there are four designs for analytical epidemiology. Ecological studies are designed to analyze associations between disease frequency and disease occurrence on a population level. A typical example is a study by Armstrong et al. (2), that uncovered a correlation between per capita fat consumption in various countries and the breast cancer mortality rate. They found higher mortality rates from breast cancer in countries with high fat consumption and a statistically positive correlation between them. A limitation of this study, however, is the presence of the bias called “ecological fallacy”. This bias means that a relationship observed on a population level does not necessarily mean a causal relation on the individual level.

Cross-sectional studies are a method of studying an association between the frequency of certain characteristics (e.g., dioxin levels in breast milk) and certain health problems (e.g., serum thyroxine levels in infants) at any given time within study groups. Temporal associations between previous exposure and present occurrence of a disease cannot be evaluated by this method.

Case-control studies are useful for testing hypotheses by comparing frequency of exposure to EDCs in an affected group and an unaffected (control) group. Case-control studies are easy and inexpensive to conduct, but there are various kinds of biases which distort true associations.

Cohort studies are designed to compare future occurrence of a disease in subjects exposed and unexposed to certain factor(s), and the groups referred to as cohort groups. In EDC studies, however, cohort groups are generally reconstructed in the past and followed up to the present. Such studies are known as historical or retrospective cohort studies.

Table 1 Types of epidemiological study

<table>
<thead>
<tr>
<th>1. Observational studies</th>
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<tbody>
<tr>
<td>1) Descriptive</td>
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<tr>
<td>2) Analytical</td>
</tr>
<tr>
<td>(1) Ecological studies</td>
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<tr>
<td>(2) Cross-sectional studies</td>
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<tr>
<td>(3) Case-control studies</td>
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<td>(4) Cohort studies</td>
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2. Intervventional studies

1) Clinical trials
2) Field trials
3) Community trials

Note: From the classification by Japan Epidemiological Association
EPIDEMIOLOGICAL REPORTS ON THE EFFECTS OF EDCs ON HUMAN HEALTH

Descriptive Epidemiology: Male Reproductive Health

Carlsen et al. (4) conducted a meta-analysis (a statistical method for combining the results of different studies (10)) of reports published between 1938 and 1991. This is the so-called "Skakkebaek report". They found a significant decrease in mean sperm counts, from $113 \times 10^9/\text{mL}$ in 1940, to $66 \times 10^9/\text{mL}$ in 1990, and in semen volume, from 3.40 mL to 2.75 mL.

The validity of the results must be discussed, however. They mentioned possible biases, such as methodological and selection biases. They only collected data from populations in different geographical regions at different times, and for different reasons. One of the most crucial problems is the two clusters of groups in their original Fig. 1: one group from 1930 to 1960, and the other from 1970 to 1990. In fact, only 12% of the subjects were from the first 30 years (14), and thus their use of linear regression analysis can be criticized. Second, they commented that the decrease in sperm count was correlated with increases in the incidence of certain genitourinary abnormalities such as testicular cancer, cryptorchism, and hypospadias. This comment is apparently based on findings regarding relationships on the population level. Since they did not analyze the relations on the individual level, their inference may impair formation of a working hypothesis.

Ecological Correlation Study: Neonatal Mortality and Dioxins from Incineration Facilities

We received a letter from an investigator who was very concerned about the human effects of dioxins discharged by incineration facilities in Saitama Prefecture, Japan. The letter was accompanied by the results of the writer's study.

He requested our comments on his findings of an ecological correlation between neonatal mortality rates and the amount of industrial wastes for incineration in Tokorozawa City, Saitama Prefecture. The amount of industrial wastes per day in Tokorozawa City increased over a period of about 30 years (1970-1996), and the decreases in neonatal mortality rate in Tokorozawa City were delayed in comparison with the decreases in Saitama Prefecture as a whole during this period. There was a correlation between them, as though the dioxins from incinerated wastes had caused the delay in the decrease in neonatal mortality rates where incineration facilities were located. Since there is a possible risk of ecological fallacy, we commented that further studies were needed to test the hypothesis on the individual level.

Cross-sectional Study: Dioxin in Breast Milk and Thyroid Hormone Levels of Infants

Nagayama (12) examined the 2,3,7,8-tetrachlorinated dibenzo-p-dioxin (TCDD) levels (ng/kg b.wt.) in the amount of breast milk consumed by infants in relation to their serum thyroxine levels ($\mu g/dL$), and found that serum thyroxine levels of the infants correlated negatively with the levels of 2,3,7,8-TCDD that they were exposed to the breast milk. This is a typical cross-sectional study suggesting that 2,3,7,8-TCDD disrupts endocrinological function.

This finding was presented at an international conference on dioxin studies in Stockholm in 1998. According to Nakarishi’s review, entitled “False clamor against dioxins (13)”, Nagayama ruled out an association because of statistical insignificance detected later. The association was considered to be a chance event or simply due to an inadequate numbers of samples (low statistical power). Further studies will be needed to determine the truth.

Case-Control Study: Dioxins and Breast Cancer

Hardell et al. (8) conducted a case-control study to test whether dioxins are related to the etiology of breast cancer. They sampled 22 patients with histologically confirmed breast cancer and 19 controls. The control group was a so-called “hospital control”. The controls were patients without breast cancer who were operated on for benign breast diseases during the same period. Dioxins were analyzed in resected tissue samples free of disease. Table 2 shows the dioxin concentrations in the case and control groups. The median concentrations of 2,3,7,8-TCDD were 3.6 pg/g and 3.3 pg/g lipid extracted from breast tissue in the cases and the controls, respectively, and the difference between them was not statistically significant. By contrast, the concentrations of octachlorinated dibenzo-p-dioxin (OCDD) were 598 pg/g, and 396 pg/g, respectively which were significantly different. The odds ratios (ORs) for OCDD were calculated with other risk factors adjusted
Table 2 Concentrations of PCDDs in 22 patients with breast cancer and 19 controls with benign breast diseases

<table>
<thead>
<tr>
<th>PCDDs</th>
<th>Cases</th>
<th>Controls</th>
</tr>
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<tbody>
<tr>
<td>2,3,7,8-TCDD</td>
<td>3.6 (1.0-7.9)</td>
<td>3.3 (1.1-6.3)</td>
</tr>
<tr>
<td>OCDD</td>
<td>598 (170-14,880)</td>
<td>396 (103-1,847)</td>
</tr>
</tbody>
</table>

Note: 1) Values are expressed as medians and range of concentrations (pg/g lipid extracted from breast tissue).
2)PCDDs: polychlorinated dibenzo-p-dioxins
3)TCDD: tetrachlorinated dibenzo-p-dioxin
4)OCDD: octachlorinated dibenzo-p-dioxin

Modified from Hardell et al. (Ref. 8)

by multivariate logistic regression analysis (Table 3). The OR was 3.8 in the 401-1,000 pg/g group and 5.2 in the >1,000 pg/g group, when the OR in the = < 400 pg/g group was 1.0.

We must point out several major epidemiological limitations. First, the comparability of the case and control groups should be taken into consideration. If benign breast diseases share the same causal factor(s) as breast cancer, the case-control study does not reveal the true risk factors of breast cancer. In an epidemiological sense, this limitation is caused by a selection bias. Second, it is also necessary to examine other risk factors that are etiologically involved in the development of cancer. Such factors are called “confounding factors”. Of course, they controlled for the factors of age, family history, body mass index, parity, age at birth of the first child, and menopausal status. It is also important to know how they collected the information from the patients, because information bias may occur during interviews. Third, OCDD must precede the development of breast cancer. A temporal relationship should be carefully evaluated from the standpoint of the pathogenesis of breast cancer.

Cohort Study: The Seveso Studies

Bertazzi et al. (3) reported the Seveso studies on effects of dioxin exposure. In 1976, an accident occurred in Seveso, Italy, in which a large population living near an agricultural manufacturing plant was exposed to 2,3,7,8-TCDD. The population’s mortality was examined during the period of 1976-1991, and the report provided scientific clues to the effects of EDC on human health.

Table 3 Odds ratios after the adjustment of other risks for breast cancer

<table>
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<tr>
<th>OCDD (pg/g)</th>
<th>Odds ratio (95%CI)</th>
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<tr>
<td>≤400</td>
<td>1.0</td>
</tr>
<tr>
<td>401-1,000</td>
<td>3.8 (0.4-39)</td>
</tr>
<tr>
<td>&gt;1,000</td>
<td>5.2 (0.4-72)</td>
</tr>
</tbody>
</table>

Note: Modified from Hardell et al. (Ref. 8)

Based on their report, we modified the author’s tables to examine the effects of 2,3,7,8-TCDD on so-called “hormone-related cancers”. We extracted six cancers: breast, genitourinary, uterus, ovary, prostate and thyroid, and established two exposure categories: high and medium exposure areas, as opposed to their three: high, medium, and low.

After our modification, Table 4 demonstrates no significant ORs, since the 95% confidence interval included 1.0, which is the risk in the reference population. Apart from the aims of this paper, the mortality from some neoplasms was related to exposure to 2,3,7,8-TCDD. The details are shown in the footnote of Table 4.

Cohort studies are very laborious in terms of the large number of data, lengthy follow-up period, and high research costs. Since they stated that the results could not be considered as conclusive, they had taken various limitations into consideration in regard to implications of their findings. Those who are interested are advised to refer the original article.

Intervention Studies

To our knowledge, no intervention studies in the strict sense described the chapter “Types of Epidemiological Studies” have ever been conducted.

EPIDEMIOLOGICAL LIMITATIONS AND CLUES TO THEIR SOLUTION

Limitations Encountered in the Epidemiological Studies on EDC

There is no question that EDCs contaminated the environment worldwide. However, hardly any information on the level of exposure to EDCs on the individual level has been obtained. Without information on current individual exposure, risk assessment for an analytical epidemiological study is impossible. Studying biomarkers of
EDCs in the human body is therefore a priority task.

Second, the interval between exposure to EDCs and the manifestation of disease is generally not well understood. It is essential to understand the natural history of disease from various aspects before undertaking EDC studies.

Third, most of the disorders under consideration are thought to be of multifactorial origin. When considering the effects of EDCs, we should exclude involvement by other risk factors and take into account interactions between EDCs and other factors. Interactions between EDCs are another issue to be discussed.

First and foremost, however, it is necessary to identify the effects of a single EDC on human health.

**Clues to the Identification of the Effects of EDC on Human Health**

As mentioned above, risk assessment on the individual exposure level is helpful in analyzing causal associations between EDCs and diseases more directly. We propose the use of cases due to accidental or industrial exposure, in which risk assessment is easy. There have been several cases that have proved useful for epidemiological studies. For example, there have been reports of cohort studies of an oil-induced disease (yu-sho) and both abnormal cognitive development (5) and thyroid function (11). There is a report of a decrease in sperm count among male workers exposed to kepone (chlordecone) or dibromopropane (7). We advise assessment of the health status of employees working in incineration facilities or in factories manufacturing EDC-related chemicals, particularly in Japan.

**Personal Comments**

Based on our 16-year study (19, 20) that identified a risk factor for the gallbladder cancer which used to be very prevalent in rice-producing areas in Japan, we would like to comment on the role of epidemiologists.

Epidemiologists must prioritize the objects of their research. Highly toxic chemicals that may cause serious health problems should be listed at the top. A “study on flea testicles” is of little value.

Epidemiologists should always take responsibility for the results that they report in public. They should not point to treasures buried in the earth, but make an effort to discover the treasures by themselves.

The total budget for research grants for EDC studies in Japan was reported to be approximately 730 million dollars in 1998 alone (1). Despite these great expenditures, the research projects have been conducted separately in each ministry in accordance with the traditional practice of vertical administration in Japan. It is no exaggeration to say that epidemiologists themselves are imprisoned within each administrative division. It seems very difficult for them to take an objective view of the problem and contribute much to the public welfare independent of any benefit that might accrue to the ministry to which they belong.
As a concluding remark, what is needed in Japanese society is not an emotional internecine battles, but a debate to ensure that we can live together on this small archipelago, with its small ecosystem.

REFERENCES