



INTERFACE:

GENES AND THE ENVIRONMENT

CENTER FOR ENVIRONMENTAL GENETICS UNIVERSITY OF CINCINNATI SPRING 1994

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Dioxin: Toxicity and Cancer

On 10 July 1976 an explosion at a chemical plant caused a cloud over Seveso, Italy, 13 miles north of Milan. The chemical plant was synthesizing a disinfectant chemical from chlorinated phenols; a by-product of this reaction is 2,3,7,8-tetrachloro-dibenzo-*p*-dioxin (TCDD; dioxin). Within a few hours, a mist began settling on the streets and buildings of the small village. Children who played in the cloud closest to the chemical plant (because it looked like a “snowy winter day”) developed phenolic burns on their skin, and some who were hospitalized exhibited abnormal liver function studies. Some children, but not others having the apparently same amount of exposure, developed *chloracne*, which resembles a cystic acne-like condition of the skin (due to proliferation of the sebaceous gland epithelium) and is a hallmark symptom of TCDD occupational exposure. Seventeen days following the accident, Italian officials confirmed dangerous levels of dioxin and ordered an evacuation. The area was subdivided into zone **A** (heaviest TCDD soil contamination), zone **B** (intermediate), and zone **R** (low).

Many of those exposed have been followed ever since. In 1989 in the *American Journal of Epidemiology*, a research team headed by University of Milan epidemiologist P. A. Bertazzi reported increased mortality from cardiovascular causes among Seveso residents between 1976 and 1986. In the September 1993 issue of *Epidemiology*, Bertazzi’s group described an elevated risk of several types of cancers for those living in the contaminated

regions of Seveso. Bertazzi’s team compared the 1976-86 medical records of 37,000 people (99% of those exposed) with those of 182,000 people in uncontaminated regions surrounding Seveso. Data in **zone A** were difficult to interpret because of the small number of people, compared with those in **zones B** and **R**. Although there was no significant increase in overall cancer risk among those exposed, elevated rates of specific types of cancers in certain subpopulations were noted: among 4,800 people in **zone B** women were five times more likely to develop gall bladder and bile duct carcinoma, compared with the control group living in the dioxin-free area; **zone B** men were twice as likely to have leukemias and lymphomas, compared with the dioxin-free control group; among 32,000 people in **zone R** men were three times as likely to develop soft-tissue sarcomas. Interestingly, these types of cancers are very similar to what has been reported in several studies of workers occupationally exposed to dioxin.

The biggest question about the data, however, is that these excess risks have been found within a decade of the Seveso accident. Most cancer scientists would agree that the usual time between occupational exposure and appearance of cancer is in the range of 15 to 30 years. One possible explanation is that TCDD is a tumor promoter for cells that have already been initiated by other agents (food, environmental chemicals, irradiation, etc.). If this is the case, these 1976-86 data are consistent with the possibility that dioxin is operating in humans as a **tumor promoter** rather than **tumor initiator**. Dioxin in numerous laboratory animal studies has not been found to be mutagenic or genotoxic, but rather a very potent tumor promoter.

The most likely reason for TCDD not being genotoxic is due to its virtual absence of being metabolized. Thus, the chemical does not form adducts with DNA and, because of a very low rate of metabolic breakdown, remains exceptionally potent in

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living organisms for a long period of time (*e.g.* years). Dioxin is known to bind to an endogenous receptor, the **aromatic hydrocarbon-responsive (Ah) receptor**, whose endogenous ligand is presently unknown. It is likely that the endogenous ligand(s) for the Ah receptor are important in various cell type-specific differentiation and growth signal transduction pathways. Dioxin can displace the putative endogenous ligand from the Ah receptor (*Figure 1*) and act as either an agonist or an antagonist, resulting in: **(a)** perturbation of differentiation (leading to birth defects); **(b)** programmed cell death of immature T cells (leading to wasting away of the thymus); or **(c)** cell type-specific proliferation (causing hyperkeratosis, chloracne, tumor promotion). This latter property of TCDD-induced growth of particular cell types is likely to be related to a report in *Fundamental and Applied Toxicology* (November 1993) which demonstrated that endometriosis is linked to dioxin exposure in monkeys. Endometriosis is a disease in which tissue (endometrium and blood vessels) from the uterus mysteriously migrates to the abdomen, ovaries, bowels or bladder and often causes internal bleeding, infertility and other problems; the etiology is unknown. Given this new information from monkey studies, epidemiologists are currently looking at endometriosis in women from **zones A, B and R** in Seveso, Italy.

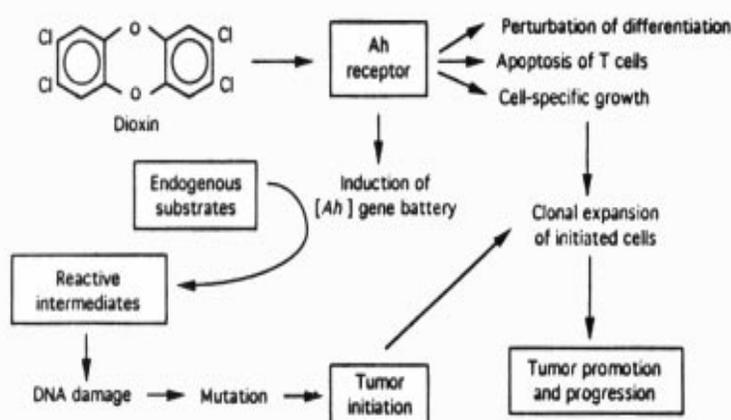


Figure 1

In unrelated research, thalidomide, a potent teratogen in humans, has recently been found to stop abnormal growth in blood vessels of the eye. Thalidomide in this instance appears to be an **antagonist** by preventing **angiogenesis**, the process by which blood vessels develop and grow. In causing endometriosis, can we presume that dioxin, also a potent teratogen, is acting as an **agonist** in promoting blood vessel growth? Could it be that this action of dioxin is also linked to its association with increased human mortality from cardiovascular disease, as described above?

Benzo[a]pyrene (BaP) and numerous other chemicals in cigarette smoke and formed during combustion, on the other hand, can act by at least two distinct pathways. **(a)** BaP is **genotoxic** and is well known to bind to the Ah receptor, thereby inducing the metabolism of BaP, which in turn leads to enhanced DNA adduct formation, mutations, and tumor initiation. BaP also induces its own metabolism. **(b)** BaP and other polycyclic hydrocarbons of combustion processes also are **nongenotoxic**, acting by one or more signal

transduction pathways. One of the most well studied of these is the action via the Ah receptor, causing cause birth defects, immunosuppression, and cell type-specific cell proliferation—most likely similar to the pathway used by TCDD.

It has been suggested by Bruce Ames and others that our bodies might receive about 10^5 oxidative hits/cell/day, meaning that dietary substances as well as environmental chemicals can be responsible for oncogene activation. Therefore, for anyone past 20 or 30 years of age, hundreds or thousands of cells in many of our tissues may already be “initiated.” As long as these initiated cells are not allowed to expand clonally, no tumor will occur. The same is true in a cigarette smoker. Although he/she might have a very large number of initiated cells, if their clonal expansion is slowed or prevented by the cessation of smoking, cancer progression is less likely to occur. Hence, if a 40-year-old smoker who has smoked 2 packs a day since age 15 does not smoke for the next 20 years, his risk of bronchogenic carcinoma at age 60 is considerably lower than if he had continued to smoke. These findings suggest that **tumor promotion** might be considerably more important than **tumor initiation** in the development of cancer. Is dioxin a human carcinogen? If dioxin is a potent tumor promoter, then, yes, dioxin might play a critical role in the progression of human cancer.

About 7 of 100 cigarette smokers die of lung cancer. Why do most smokers not die of bronchogenic carcinoma? CEG researchers have recently determined the DNA differences between mice with the high-affinity Ah receptor gene (Ahr^{b-1}) and mice with the low-affinity Ah receptor gene (Ahr^d). Years ago the **b-1/b-1** mice were shown to be 3- to 20-fold more prone to cigarette smoking-induced lung cancer than **d/d** mice. Interestingly about one-tenth of the human population exhibits the high-affinity Ah receptor phenotype. If data from different strains of mice can be extrapolated to humans, this suggests that this one-tenth of the human population might be at greater risk than the other nine-tenths of the population, for bronchogenic carcinoma among cigarette smokers--- given the identical amount of exposure to cigarette smoke and other combustion processes. The same might be true for other types of cancer (especially of the head and neck) among cigarette smokers. How much of this greater risk due to the high-affinity Ah receptor is due to tumor initiation, and how much is due to tumor promotion, remains to be delineated. Are the high-affinity Ah receptor humans more likely to develop chloracne than the low-affinity Ah receptor individuals, given the same exposure to dioxin? The answers to some of these questions are being sought by investigators in our Center for Environmental Genetics ---*Contributed by Daniel W. Nebert*

CEG Members in the News

The participation of CEG members who have provided information on their activities over the last few months is much appreciated. Please continue to do so and, to any members not participating in this issue, please do not hesitate to offer information in the future!

Eula Bingham was a panel member for the NIH Technical Assessment Workshop on "The Persian Gulf Experience and Health," run by the NIH Veteran Affairs Administration (Bethesda, Maryland), spring 1994. She was also awarded the American Conference of Governmental Industrial Hygienists (ACGIH) 1994 William Steiger Memorial Award during the Annual American Industrial Hygiene Conference and Exposition (AIHCE) meeting (Anaheim, California), May 1994.

Kathleen Dixon was invited to speak at a session on "Problems and Progress in Deconvoluting Mutation Spectra," at the 22nd Annual Meeting of the American Society for Photobiology (Scottsdale, Arizona) and to give a lecture entitled, "UV mutagenesis in mammalian extracts," at the 1994 Gordon Research Conference on Mutagenesis (Plymouth, New Hampshire), both in June 1994.

George Leikauf gave the plenary lecture entitled, "Evaluation of a possible association of air toxics and asthma," at the workshop on "Air Toxics and Asthma: Impacts and Endpoints," presented by the Mickey Leland National Urban Air Toxics Research Center (Houston, Texas), February 1994.

Grace Lemasters was chair of a session, "Reproductive Surveillance in the Workplace" and gave a talk entitled, "The nuts and bolts of implementing a reproductive surveillance program," at the American College of Occupational and Environmental Medicine (Chicago, Illinois), April 1994.

Gordon Livingston presented a paper entitled, "Radiobiological effects in the population living near Chernobyl," at the Annual Environmental Mutagen Meeting (Portland, Oregon), May 1994.

Dan Nebert has been invited to organize and co-chair a symposium on "Signal transduction and drug metabolism," at the 7th Annual Meeting of the International Union of Pharmacology (IUPHAR). Five speakers participated in this symposium on "Signal transduction and drug metabolism" (Montreal, Canada), July 1994. The title of his talk is "Evolutionary argument for the relationship between signal transduction and drug-metabolizing enzymes."

Steve Potter spoke on "Genetic circuitry of mammalian development," in the Department of Cell Biology and Anatomy at Cornell University (New York), April 1994.

Alvaro Puga was invited to present a talk entitled "Activation of intermediate-early proto-oncogenes by polycyclic aromatic hydrocarbons," at the Centre de Medicaments, University of Nancy-I (Nancy, France), April 1994.

Carol Rice was invited to present a talk on "Use of employee interviews and faculty engineering data to predict and evaluate changes in airborne fiber concentration in RCF manufacturing," at the Conference on Retrospective Assessment of Occupational Exposures in Epidemiology (Lyon, France), April, 1994.

Wilson Tabor lectured on "Laboratory accreditation systems" and "Laboratory quality assurance and control in the public health system," at the Instituto Nacional de Salud Publica (Cuernavaca, Mexico), April, 1994. He is also developing an Environmental Health and Safety Program for the Mexican Ministry of Health, for which he has been an external scientific advisor since 1989.

Glenn Talaska has been invited to present a lecture, "Molecular biomarkers of occupational lung carcinogen exposure," at the Annual Occupational Health Seminar sponsored by The Institute for Occupational Health, Yonsei University School of Medicine (Seoul, Korea), August 1994.

David Warshawsky was invited to present a seminar "Metabolic activation of environmental carcinogenic N-heterocyclic aromatics: Metabolism, DNA binding and biological consequences," for the Joint Graduate Program in Toxicology, Environmental and Occupational Health Sciences Institute (EOSHI), Rutgers University (Piscataway, New Jersey), April 1994.

LETTERS

TO THE EDITOR:

Dan, many congratulations on the first issue of *Interface*, which arrived on my desk today (19 January 1994). I hope that you will be able to sustain the momentum! Under the address at the end of the Newsletter, you might consider giving a fax number and E-mail address to encourage easy and quick correspondence or comments.

---Jeffrey R. Idle
Editor, Pharmacogenetics
University of Newcastle-upon-Tyne
NE2 4HH, United Kingdom

RESPONSE FROM THE EDITOR

Thank you for the compliment, Jeff! Also, your suggestion is an excellent one, and, as you can see, we have included our fax numbers and my email address in this issue. -DWN

Q How did you select the subject of the Gulf War Syndrome for your first issue? Many people feel that chemical warfare is perhaps the least likely to be relevant to this illness.

A The purpose of our *Interface* newsletter is to provide a forum for discussion, among professional and semi-lay individuals and groups, of the “interaction between genes and the environment.” One way to start the ball rolling in the first few issues of the newsletter is to write provocative commentaries and prose penetrating questions on timely matters of public concern, which have appeared in the media, are somewhat controversial, and involve public health policy, environmental genetics and toxicology. Such commentaries might result in (a) our encouraging colleagues to carry out definitive experiments in order to prove or disprove the provocative hypotheses, or (b) in readers writing letters to the editor and citing publications or otherwise proving that I am definitely on the wrong track. --DWN

Q Has human serum paraoxonase been correlated with organophosphate sensitivity in vivo?

A To date, serum paraoxonase activities have not been directly correlated with organophosphate sensitivity in humans in vivo. However, this correlation has been demonstrated in the rabbit in vivo [*Drug Metab Disp* 13: 640-645, 1985; *J Toxicol Environ Health* 40: 337-346, 1993], birds in vivo [*J Toxicol Environ Health* 40: 337-346, 1993] and human serum in vitro [*Banbury Report* 16: 167-178, 1984; *Drug Metab Disp* 12: 57-62, 1984; *J Toxicol Environ Health* 40: 337-346, 1993]. Of course, a clinical study with organophosphate insecticides would be unethical, but, should there occur an accidental environmental exposure of a group of workers to one or another organophosphate, this would be an excellent opportunity for human geneticists/epidemiologists to try to correlate the intensity of the toxic response with the paraoxonase (PON) genotype.

Incidentally, in the press in recent weeks it has been revealed that Persian Gulf War troops were given a drug intended to counter Iraqi chemical or biological weapons. Now some are questioning whether the side-effects of this drug are causing the illness that thousands claim to be

suffering. The Food and Drug Administration approved the Defense Department's widespread distribution of the drug, *pyridostigmine*, an antidote of anticholinesterase poisoning. Quite likely, variability observed in the responses to receiving *pyridostigmine* would be based on allelic differences in the *PON* gene. ---DWN

Q There is some confusion about the factual details of the story about the Gulf War Syndrome. These were actually sailors (Sea Bees), not soldiers, who were exposed and began to get sick. Where did you get the number “8,000 military personnel who have become sensitized.”

A I stand corrected on the factual details about who, and how many, military personnel were exposed. My only source was *Time Magazine* [p. 43, Nov. 22, 1993].-----DWN

Q How can you accept a multiple chemical sensitivity (MCS) that is so indiscriminate with respect to chemical identity? And would it have an enzymatic basis, or an immunological basis?

A There is so little known about the true etiology of MCS. My feeling is that other medical entities -- such as asthma/bronchial hyperreactivity, “sick building syndrome,” and “food intolerance syndrome” --overlap with MCS in that one particular stimulus might have initially provoked the immune response, but then other stimuli (seemingly quite unrelated in chemical size or structure) might also become able to provoke the same response. The etiologies might easily include, at least in part, a combination of an enzymatic and an immunological basis. For example, antibodies to cytochrome P450 CYP2D6 and several other drug-metabolizing enzymes, have been found in pediatric autoimmune hepatitis, but whether this is cause or effect remains unknown. The *NAT2* slow acetylator phenotype has been shown to have a much higher risk of systemic lupus erythematosus than the *NAT2* rapid acetylator phenotype. It is certainly feasible that covalent binding of reactive metabolites might produce the haptene, or whatever other type of antigen is necessary, to initiate unknown process(es) in producing the aberrant immune response. Finally, the systemic nature of the above-mentioned immunologic-like responses suggests to me that ubiquitous cells, such as macrophages or white blood cells, and ubiquitous cytokines are likely to be mediators of the response --- DWN

The Care and Feeding of Tissue Culture Cells

1 Cells need to be fed: if they are not fed, they will die. Cells in general grow very well in medium containing 5% serum, but the medium must be changed every 3 - 7 days; otherwise, the cells will round up, detach from the flask, and die. All those round objects that one sees in a flask after 5 - 10 days without changing the medium are dead cells.

2 The more cells in a flask, at least initially, the faster the cells grow; the more frequent the medium changes, the faster the flask reaches the stage of confluence.

3 The medium contains serum; the serum contains growth factors which make cells grow. Several considerations are pertinent:

a. Growth factors are very fragile substances. They self-destruct in a relatively short period of time. That is one reason cells must be refed periodically, to replenish them (the other reason being that the cells themselves use up the growth factors). Since these factors are labile, if you place your medium in a 37° C water bath at 9:00 a.m., leave it there for eight hours and use it at 5:30 p.m., you have unwittingly contributed to the depletion of needed factors from your medium. Consequently, the cells will die faster than expected.

b. Growth factors are important regulators of cell physiology and, therefore, are important determinants of the outcome of your experiments. It is essential that experimental conditions take this fact into account. Growth factors become depleted with time and we have no idea of how they affect our experiments. The experimental parameters must stay the same: cells always fed 4 hr. before the experiment, or the experiment always done in spent medium the third day after feeding. At least, be consistent. Otherwise, do not be surprised if your values are "10 and 100 units" in one experiment and "50 and 300 units" in the next experiment.

c. Remember that fetuses have more cytokines than babies, and babies more than young. So, if you suspect a cytokine effect, use calf serum instead of foetal serum. Never change from one to the other and expect the same results. Remember that the

cows in Colorado eat differently, have a different everything, than the cows in Maryland. The serum from cows eating hay in January will differ from the serum from cows eating fresh clover in June. No two sources of serum are alike. When in doubt, carry out your experiments in 0.1% serum medium.

4 Some cells can take up to a 1:80 split, while others might need a 1:4 or 1:8 split. Cells will grow slowly if plated too thinly. When seeding cells, you must spread them out well into the flask or plate. Two things are essential for a good spread: first, make sure you have good single cell suspensions; second, distribute the cells well when you seed them. For good single cell suspensions, trypsinize the cells and then pipette them up and down several times, pressing the pipette against the bottom of the flask so that you feel resistance to the flow. For good distribution, always follow the rule of motions; swirl the seeded plate 10 times clockwise, 10 times counter-clockwise, 10 times back and forth, 10 times sideways. Then repeat the process. What is important is that the cells are properly seeded, not that you go through the motions. Therefore, after plating, look. Put the plate under the microscope and look. If it isn't good, make it good---do it again.

5 Remember that poorly designed or executed experiments are only a waste of time and resources. Everybody makes mistakes, but not everybody learns from them. -Contributed anonymously

GOODBYE TO A WONDERFUL PERSON

Lewis Thomas, 80, Harvard-trained physician, father of modern immunology and experimental pathology, poet-philosopher of medical science, died December 3, 1993. Professor Thomas gained worldwide public acclaim when his essays (from his column in the New England Journal of Medicine) were collected in prize-winning books, "The lives of a Cell" and "The Medusa and the Snail." Author of more than 200 scientific papers and a total of six superb books, he proposed the novel idea in 1959, that tissue histocompatibility proteins (responsible for graft rejection) constitute a means to defend the body against cancer. By the end of this year, a Lewis Thomas Chair will be established at Cornell University Medical College, New York City.

RECENT CEG-SPONSORED SPEAKERS

JANUARY 5, 1994

Richard D. Irons, Ph.D.
Director, Molecular Toxicology and Environmental
Health Sciences Program

"Of mice and men: Studies on the mechanisms of chemical leukemogenesis"

APRIL 1, 1994

Gerald V. Poje, Ph.D.
National Institute of Environmental Health Sciences
Bethesda, MD 20892

"Environmental equity"

APRIL 13-14, 1994

Irwin Fridovich, Ph.D.
James B. Duke Professor
Department of Biochemistry
Duke University Medical Center
Durham, NC 27710

"The two faces of oxygen"
"Problems imposed by redox-cycling compounds and the adaptive responses thereto"

MAY 11-12, 1994

Arno G. Motulsky, M.D.
Professor of Medicine and Genetics
Department of Medicine
University of Washington
Seattle, WA 98195

"Genetics and environmental disease"
"Pharmacogenetics and ecogenetics: Models for genetic susceptibility to common diseases"

MAY 17, 1994

Johannes Doehmer, Ph.D.
Technische Universität München
Institute für Toxikologie und Umwelthygiene
80636 Munich, Germany

"V79 Chinese hamster cells genetically engineered for cytochromes P450 and the inducible NO-synthase, and their application in toxicology and pharmacology"

CEG Round-table discussion on molecular and genetic epidemiology

May 23, 1994

"Incorporation of genetic information into epidemiological studies"

Discussants:

Robert L. Bornschein, Ph.D. (UC, Department of Environmental Health)

Janet Elashoff, Ph.D. (UCLA and Cedars Sinai Hospital)

Michael Elashoff, Ph.D. candidate (Harvard School of Public Health)

Joanna Groden, Ph.D. (UC, Department of Molecular Genetics)

Vicki S. Hertzberg, Ph.D. (UC, Department of Environmental Health)

INTERFACE is supported by NIH grant # ES06096 from the National Institute of Environmental Health Sciences, and is published by the University of Cincinnati Center for Environmental Genetics, Daniel W. Nebert, M.D., Director

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INTERFACE

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