Letters

Love Canal Chromosome Study

I have had the opportunity to examine the photographs of chromosomes in 151 metaphase spreads (and 111 accompanying karyotypes) from short-term lymphocyte cultures of Love Canal residents prepared by Dante Picciano, scientific director of the Biogenics Corporation, for the Environmental Protection Agency (EPA). In addition, I have examined 200 dividing cells under the microscope from four previously unscored slides prepared for the study. Picciano's report to EPA was sharply criticized in Gina Bari Kolata's article "Love Canal: False alarm caused by botched study" (News and Comment, 13 June, p. 1239).

I would like to comment on four of the controversial issues that have arisen from Picciano's pilot study. These are (i) the quality of the cytogenetic preparations, (ii) the cytogenetic interpretation of the abnormalities reported, (iii) the lack of simultaneous controls, and (iv) the biological significance of chromosomal abnormalities in terms of health effects such as cancer and birth defects. A detailed report of my observations will be sent to EPA and will be published in the *Mammalian Chromosome Newsletter*.

Although Picciano and I both live in Houston, I had never met him until 12 June 1980. I was in Australia and New Zealand from 9 May until 1 June and was unaware of the Love Canal chromosome study until I returned home. At that time Picciano offered to make the photographs and slides available to me for review.

Quality of cytogenetic preparations. I subjectively classified the quality of the photographs I examined from Picciano's 36 subjects, with 83 percent scored as good to excellent and 17 percent fair to poor. This is in contrast to the EPA-sponsored panel report (Roy Albert, chairman) which states that the quality of the Xerox copies of photographs of metaphase spreads which they examined was fair or poor. Most of the chromosomes I examined did *not* exhibit overcontraction due to excess Colcemid exposure. None of the cells showed the severe chromosome damage commonly seen in cultures exposed to clastogenic agents in vitro.

Cytogenetic interpretation. Cytogeneticists are aware of differences among observers in scoring chromosomal abnormalities. I tended to score many abnormalities as "chromatid gaps" that were scored by Picciano as "chromatid breaks." I also scored fewer "chromosome breaks" than Picciano. Nevertheless, our agreement was remarkable about other unstable abnormalities (Table 1).

Kolata states (p. 1241), "[t]he EPA panel concluded that . . . supernumerary acentric chromosomes exist only in the mind of Picciano." Because of this damning statement I wish to report my observations of Picciano's photographs containing abnormalities which I recorded as "long acentric fragments." Among 15 cells, all of which were karyotyped, I found 28 acentric fragments. Eight of these fragments were as long as the long arm of a No. 2 chromosome while nine were *longer* than any chromosome arm of the human complement. The latter could not result from a simple chromosome break. Unless they were chromosomes with centromere inactivation or premature separation of the centromere, they must represent some form of breakage and reunion. Without C-banding and G-banding the derivation of these objects remains unsettled. In nine of the 15 cells there was significant chromosomal material present in addition to the normal diploid complement, and in two other cells extra material was probably present. This was observed in cells from both males and females. Among the 200 cells examined under the microscope I scored three additional long acentric fragments.

Table 1.

Unstable chromosome abnormalities	Picciano (No.)	Shaw (No.)
Dicentrics	1	1
Chromatid interchange (triradial figure)	1	1
Rings	5	3 definite; 3 possible
Acentric "double minutes"	0	2
Long acentric fragments	14	28

In my experience, long acentric fragments are very rarely seen in normal individuals. I could find no cytogenetic surveys in which they were separately categorized and commented upon. However, there are at least two photographs published in the literature in which long acentric fragments appear (*1*). I cannot agree that supernumerary acentric fragments are a figment of Picciano's imagination.

Rings and chromatid interchanges are also rare in normal individuals. Court-Brown *et al.* reported (*2*) that *no* rings were observed in any preparations from their random sample. They examined 12,264 cells, of which 8,983 were cultured for between 65 and 75 hours, compared to 9,102 cells examined by Picciano. German (*3*) reported two quadriradial configurations in cells from 49 clinically normal individuals studied, but one of those appeared in a nonradioactive cell from a culture that had been exposed to tritiated thymidine.

The lack of simultaneous controls. The results are neither positive nor negative because of absence of contemporary controls. I find it difficult to understand why the EPA panel stated flatly that the absence of simultaneous controls was a very serious deficiency of the study and then stated that Picciano's results were considered to be well within normal limits. Were they trying to say that the study is a "false alarm" or that it is "botched" because their own conclusions are different from Picciano's, even though both used historical controls?

I believe that a sensibly designed, controlled, collaborative study should be undertaken as soon as possible. Further, I suggest that the cytogenetics community attempt to design a study that would be acceptable *in advance*, considering all of the possible parameters, such as culture conditions, intraobserver consistency, interobserver differences, suitable control groups, appropriate staining procedures, number of cells per individual and number of individuals to be scored, number of laboratories, and blind scoring of subjects and controls. Until a consensus is reached concerning a research protocol and the interpretation of possible results, it is a waste of time to gather more data that cause anxiety and anguish among the Love Canal residents. Cytogeneticists could perform a useful service by designing chromosome studies of humans exposed to toxic chemicals. Undoubtedly many studies will be request-

ed in the future by government, industry, exposed populations, special interest groups, and others. Expert advice from statisticians and epidemiologists would be necessary for developing an acceptable research design.

Biological significance. It is difficult for the lay person to understand uncertainties in science, yet it is imperative that scientists attempt to educate the nonscientist concerning the problems which arise when extrapolating from laboratory data to an assessment of risk in human populations. Stochastic events that occur after exposure to mutagens, clastogens, carcinogens, and teratogens are not easy to explain. Chromosome damage is only one indicator in a series of poorly understood biological events that occur randomly in cells (and therefore in individuals) as a result of an external environmental insult. We cannot equate a ring chromosome in a lymphocyte with a cleft palate in an offspring. We should recognize our ignorance and uncertainties and try to help the regulators as well as the human subjects to appreciate the concept of probabilities rather than certainties. In our democratic society, perhaps we will decide that 500,000 deaths per year is an acceptable price for toxic chemicals in our environment, just as we have decided that 50,000 traffic deaths per year is an acceptable price for automobile travel. On the other hand we may decide that 5000 deaths per year is an unacceptable price for toxic chemicals. The scientists should provide the data and interpret the results; the public should decide.

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References

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2. W. M. Court-Brown, K. E. Buckton, P. A. Jacobs, I. M. Tough, E. V. Kuenssberg, J. D. E. Knox, *Eugen. Lab. Mem.* **42**, 35 (1966).
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Kolata's article about the pilot cytogenetic study of Love Canal residents raises some important issues. I would like to comment on some related issues and their broader significance.

I would like to confirm that the study conducted by the Biogenics Corporation did not serve as the basis of the President's decision to provide federal funding for the temporary relocation of Love Canal residents and for health and environmental studies in that area. Those actions, widely supported by most scientific administrators, were taken because of the cumulative evidence of exposure to toxic wastes and because of mounting evidence of resulting health effects.

Our primary concern in releasing the study was fairness and forthrightness in explaining the results to the 36 participants and to the other Love Canal residents. Unfortunately, the data were leaked to several newspapers before we could initiate peer review of the study. Because of the many caveats that needed to be placed on the conclusions, we went ahead with personal explanations to the participants on 15 May. That action, of course, constituted public release of the data, so we complied with news media requests for a formal briefing later in the day. In the press briefing, we carefully pointed out that scientific review had not yet been performed (a caveat largely ignored by most of the press accounts) and that such a review of the study was planned for 18 May. After that initial review was subsequently frustrated, I asked Roy Albert of New York University to assemble a panel of experts to review the pilot study and materials released to the Environmental Protection Agency (EPA) by Biogenics. The Albert review, forwarded to me on 12 June and released to the public, concluded that the study "should be regarded as indeterminate." The panel said that the "results are not positive in terms of the norms for the occurrence of chromosomal abnormalities, but the study cannot be called negative because of technical inadequacies and the lack of a control group." The panel also indicated that the "purported occurrence of a rare abnormality, 'supernumerary acentric fragment,' was not substantiated." I have accepted the findings of the Albert review and feel that little of scientific value can be gained from further rehashing of the pilot study, because of its shortcomings. For the future, EPA is working with the Center for Disease Control to undertake a comprehensive cytogenetic study of Love Canal residents.

There are well-established norms for the conduct of scientific investigations. Among these are peer review of research protocols and of research results, including full disclosure of techniques and data. EPA adheres to those norms; indeed, we have been continually strengthening our peer review mechanisms. We extensively use the agency's science advisory board to review research programs and health and environmental criteria documents. We widely use peer review mechanisms for intramural research and for extramural grants and cooperative agreement awards. Recent-

ly, we improved our grant programs by making all grants nationally competitive and subjecting the proposals to peer review, performed largely by academic experts, in a similar manner to the reviews by the National Institutes of Health. In every aspect of our scientific program, we have been diligent in making sure our practices are generally acceptable to the scientific community.

There were flaws in the conduct of the pilot chromosome study. In the normal course of events, the inadequacies of the pilot study would have been discovered and the study would have been corrected or stopped. However, in this incident, there were two additional complicating factors. First, in the urgency to prepare for litigation on Love Canal, the government scientists and lawyers involved did not raise questions about the study's design, conduct, and quality with individuals at the appropriate management level for resolution. Second, members of the press obtained the raw results of the study before EPA management received them.

On the first point we have taken a number of steps to strengthen management direction over such activities and to require adequate internal and, where appropriate, external review of all such technical proposals.

The second point raises the broader issue of the proper handling of scientific data in situations of public concern where there is an implied or expected need for immediate action. Premature releases of scientific data will, of course, confront EPA and other public health regulatory agencies in the future. Often, as will likely be the case as the new manifest system established under the Resource Conservation and Recovery Act indicates where hazardous wastes are being dumped, such releases will introduce new tensions into already anxious communities. As a rule, the affected communities are much more likely than scientists to conclude that there is a cause and effect relationship between a possible exposure and observed health problems. Public officials must deal directly and quickly with these difficult and touchy situations; however they must carefully qualify the data, recognizing that the many combinations of chemicals, exposure modes, and toxicological effects have generally pushed us well beyond the limits of scientific knowledge. They must withstand the pressure for a "bottom line" as to whether the associated risk is acceptable or whether health damage is really occurring, until adequate studies and reviews are completed. Finally, they must not withhold information or else they further damage the government's credibility with a perceived "cover-up."

We believe that improved management controls can help reduce the likelihood of such an incident recurring. However, there remains the basic conflict between the imperative "for the public to know now" in the face of perceived health problems and the necessity for adequate scientific review. This conflict will continue to be a difficult problem for both public policy-makers and scientists in the public health field.

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I would like to comment on the article "Love Canal: False alarm caused by botched study," which appeared in the 13 June issue. The cytogenetic study of the Love Canal population is totally overshadowed by the outcome of the last 18 pregnancies among the residents: two births were normal; nine children had birth defects; there were four spontaneous abortions and three stillbirths.

With regard to the pilot cytogenetic study performed by the Biogenics Corporation, I met with officials of the Environmental Protection Agency's (EPA) Hazardous Waste Enforcement Division and the Health Effects Division on 16 January 1980. I asked the Enforcement officials the following questions. "What is the question you want answered? Do you want to know if the residents of Love Canal have an increased level of chromosomal damage, or do you want to know if chemical exposures at Love Canal are causing an increase in the level of chromosomal abnormalities in the Love Canal residents?" I explained the differences in the questions to the Enforcement officials, and they told me that the Justice Department was interested in determining if the residents of Love Canal had an increased level of chromosomal damage. They wanted the results as soon as possible. We agreed on a minimum sample size of 25 residents and 25 contemporary controls.

On 18 and 19 January, the Biogenics Corporation obtained a total of 36 blood samples from the residents of Love Canal. We were not provided with contemporary controls. We discussed the control situation with an EPA attorney who assured us that controls would be made available. The Biogenics Corporation was also given slides from ten Love Canal residents from a February 1979 study. This study was conducted at Roswell Park Memorial Institute by another investigator under a different set of laboratory conditions. EPA asked Biogenics to analyze these previously unanalyzed slides.

On 23 January, officials of EPA's Office of Enforcement asked Biogenics if we would be willing to return to Buffalo to obtain blood samples from controls. We agreed, but we never received authorization from EPA. We repeatedly cautioned the Enforcement officials that, without contemporary controls, no definite conclusions could be drawn from the analysis of 36 Love Canal residents. Officials from the EPA Health Effects Division similarly cautioned the Enforcement officials.

All the slides were coded and presented to a single technician for analysis. The technician has had 16 years of experience examining slides for chromosomal aberrations. The technician was told that the slides were from both Love Canal residents and controls. For the analyses, 200 randomly selected cells were examined for each individual, except for three cases where 131, 184, and 187 cells were scored because of an inadequate number of metaphase figures available.

On 2 May, I informed the Enforcement officials that the analyses were complete and that I was concerned about eight residents with supernumerary acentric fragments, four residents with ring chromosomes, and one resident with a triradial exchange figure. In all, 12 individuals had these types of aberrations (one individual had supernumerary acentric fragments, a ring chromosome, and a dicentric chromosome). Similar analyses of the previously unscored slides from the Roswell Park study showed one of ten individuals with three supernumerary acentric fragments. We do not know for certain whether any people in the Roswell Park study were also examined in the Biogenics study, so we can only report them separately.

On 7 May, EPA officials visited our laboratory in Houston. We showed the work and explained the results to the EPA officials and cautioned that "in the absence of a control population, prudence must be exerted in the interpretation of such results." An official from the Health Effects Division expressed concern that the Enforcement officials had not authorized the contemporary controls. The Enforcement officials asked us to write up a detailed description of what we had done and to give them the most likely interpretation of what caused the reported results. We cautioned the Enforcement officials that a complete study was necessary before definite conclusions could be made about

the cytogenetic findings of the Love Canal residents. We were told that the preliminary results would only be used as part of the Justice Department's injunction procedure against the Hooker Chemical and Plastics Corporation, so that additional studies of the Love Canal residents would be required.

On 15 May, we sent the completed report, entitled "Pilot cytogenetic study of the residents of Love Canal, New York," to EPA. In the "Results" section, the report states, "Since a contemporary comparison group (control group) was not available for study, no unequivocal statement can be made concerning the cytogenetic results from the Love Canal residents." In both the "Discussion" and "Summary" sections, the report states, "However, in the absence of a contemporary control population, prudence must be exerted in the interpretation of such results." In the "Summary" and "Recommendations" sections we strongly recommended additional genetic testing in as many of the Love Canal residents as possible.

In short, we did exactly what EPA asked us to do. Complete documentation of the events through 15 May is available, since the EPA project officer kept detailed records of all meetings and telephone conversations.

On Friday, 16 May, I received a call from EPA officials informing me that David Rall of the Department of Health and Human Services (HHS) was forming a panel to visit our laboratory and review our results. I told EPA officials that the review was fine with me. I then discussed the HHS committee with officers of the Biogenics Corporation. I expressed some concern that there might be a conflict of interest on the part of one committee member because it had been reported that he was investigating the possibility of starting his own cytogenetic analysis company similar to ours. We informed EPA officials of our concern and told EPA that, if that member was removed from the committee, Rall could add anyone he wanted and, in addition, select any one of five individuals we recommended.

On Monday, 19 May, I received a call from Charles Carter, who informed me that he was acting for Rall in the selection of the HHS committee. He told me that no one had been removed from the committee, but he would add one of our five choices. We were not happy with the arrangement, but we did not object. Later on the same day, I received a call from Rall's secretary, who informed me that Carter was on his way to Houston and that the committee would be composed of eight individuals. Not one of our five nominees had been selected. We were very upset that our modest requests had not been met and told Rall's secretary to have Carter call us upon his arrival in Houston.

Late that same afternoon, we received a call from a representative of the Love Canal Homeowners' Association expressing concern about the participation of another HHS committee member because he was employed by the New York State Health Department and there might be a conflict of interest. At approximately midnight, Carter called my office, and I answered the phone. I expressed our concerns to him and asked if he would like to come to the laboratory and discuss the makeup of the committee. I told him that I would drive to his hotel and pick him up. He declined to visit the laboratory and discuss the committee.

On Tuesday morning, I received a call from Barbara Blum of EPA. She expressed concern about the immediate review of our work and asked if she could negotiate the selection of the HHS committee with us. We agreed. After repeated negotiations through Blum with the HHS committee (now made up of five reviewers and two observers), we were asked to nominate one individual. He was rejected.

The HHS committee would no longer negotiate the composition of the committee, and they decided to review our report and make their own report without visiting our laboratory or seeing our results. We announced the formation of an independent review committee comprised of three geneticists. We wanted the committee to have as many grant-independent geneticists as possible. Later on Tuesday, an EPA cytogeneticist visited our laboratory and reviewed our slides, photographs, and results from the Love Canal studies. He expressed surprise at the size of some of the supernumerary acentric fragments. He also stated that he was impressed with the quality of our work. We made photocopies of our photographs, karyotypes, and results available to him so that he could take the copies back to Washington with him.

On 5 June, the independent review committee submitted their report to the EPA. They concluded that:

1) Some of the individuals in the study had aberrations that were beyond normal limits expected in 36 healthy people.

2) The blood cultures were set up and processed in an appropriate manner consistent with standard practice in reputable cytogenetic laboratories.

3) The study revealed cytogenetic markers which included unique large acentric fragments in a number of cells which proved, after karyotype analysis, to be extra genetic material. In most cases these acentric fragments were longer than any arms of any of the chromosomes, showing that this aberration had replicated in serial cell generations. Although an induced artifact is always a possibility, the committee felt that this was highly unlikely. This conclusion was fortified by the fact that one of ten Love Canal residents involved in a previous cytogenetic study done in a different laboratory showed the same type of acentric fragment.

4) This was a pilot study and contained certain limitations, such as no control group. The review group strongly felt that these individuals should have another cytogenetic analysis with an adequate concurrent control group included in the protocol. Those with persistently high cytogenetic aberrations should undergo genetic counseling.

I cannot respond to the EPA-sponsored panel organized by Roy Albert because I have not seen the report. However, Kolata states that the EPA panel concluded "that supernumerary acentric chromosomes exist only in the mind of Picciano." In the original report to EPA, I used the term supernumerary acentric chromosomes in the "Summary" section and supernumerary acentric fragments throughout the remainder of the report. I sent EPA a corrected "Summary" calling the aberrations supernumerary acentric fragments. These supernumerary acentric fragments now exist in the minds of seven geneticists, including the chief of Genetic Toxicology for EPA.

It is common knowledge that Hooker Chemical hired a geneticist to review Biogenics' pilot study. The Hooker medical director called Biogenics informing us of their proposed review and their desire for information. It would be of interest to all if their report were made public.

On 6 June, we received a call from an EPA contracting firm informing us that the Office of Enforcement wanted additional detailed cytogenetic studies of the Love Canal residents along with appropriate contemporary controls. The EPA letter to the contracting firm was dated 20 May. To date, we have not received authorization for the additional studies. Meanwhile, the problems of Love Canal and the other 51,000 chemical dumps continue.

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