CIRCULATION COPY
SUBJECT TO RECALL
IN TWO WEEKS

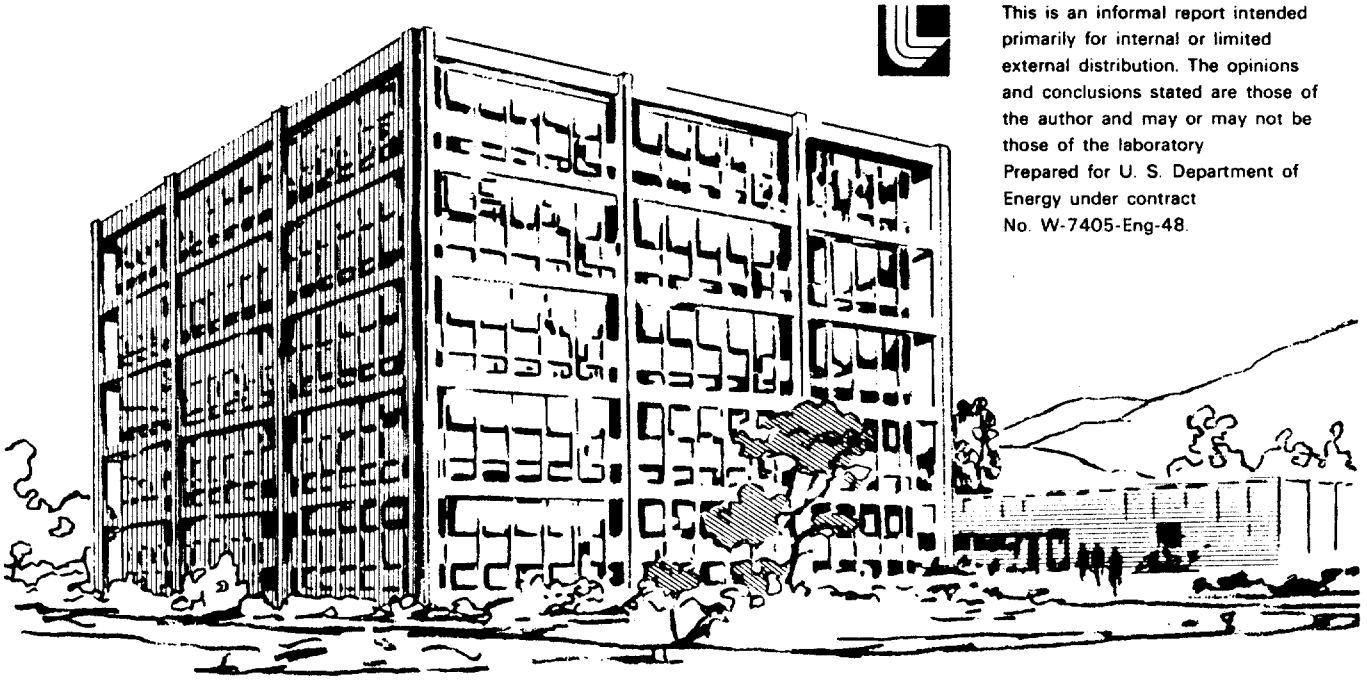
UCID- 18222-79-1

Lawrence Livermore Laboratory

SPERM SHAPE ABNORMALITIES INDUCED BY ENERGY-RELATED HYDROCARBONS AND INDUSTRIAL CHEMICALS - Progress Report - Jan. 1 to June 30, 1979

A. J. Wyrobek

July 23, 1979



This is an informal report intended primarily for internal or limited external distribution. The opinions and conclusions stated are those of the author and may or may not be those of the laboratory
Prepared for U. S. Department of Energy under contract No. W-7405-Eng-48.

DISCLAIMER

This document was prepared as an account of work sponsored by an agency of the United States Government. Neither the United States Government nor the University of California nor any of their employees, makes any warranty, express or implied, or assumes any legal liability or responsibility for the accuracy, completeness, or usefulness of any information, apparatus, product, or process disclosed, or represents that its use would not infringe privately owned rights. Reference herein to any specific commercial product, process, or service by trade name, trademark, manufacturer, or otherwise, does not necessarily constitute or imply its endorsement, recommendation, or favoring by the United States Government or the University of California. The views and opinions of authors expressed herein do not necessarily state or reflect those of the United States Government or the University of California, and shall not be used for advertising or product endorsement purposes.

This report has been reproduced
directly from the best available copy.

Available to DOE and DOE contractors from the
Office of Scientific and Technical Information
P.O. Box 62, Oak Ridge, TN 37831
Prices available from (615) 576-8401, FTS 626-8401

Available to the public from the
National Technical Information Service
U.S. Department of Commerce
5285 Port Royal Rd.,
Springfield, VA 22161

SPERM SHAPE ABNORMALITIES INDUCED BY ENERGY-RELATED HYDROCARBONS AND INDUSTRIAL CHEMICALS

Old Title: Morphological Variants in Damaged Sperm

EPA-IAG-D6-E681-AN

Progress Report

Period covered: January 1, 1979 to June 30, 1979

Principal Investigator

Andrew J. Wyrobek
Lawrence Livermore Laboratory
University of California
P.O. Box 5507
Biomedical Sciences Division
Livermore, California 94550

Project Officers

DOE: David A. Smith
DOE, ASEV/OHER
Washington, D.C. 20545

EPA: Gerald J. Rausa
EPA/ORD/OEMI
401 M St. S.W.
Washington, D.C. 20545

OUTPUT TITLE: Using existing and new biological screening and testing systems, identify the presence of carcinogenic, mutagenic, teratogenic and physiologic or metabolically toxic agents associated with coal and oil shale extraction, conversion or utilization.

AIM: To further develop and apply the detection of morphologically abnormal mammalian sperm as a rapid, simple, quantitative assay of the pathologic response of the male gonad to toxic agents associated with the recovery, process stream, and emission of nonnuclear sources of energy, with primary attention to substances from *in situ* coal gasification and *in situ* coal-shale extraction.

I. Work Accomplished: January 1, 1979 to June 30, 1979

A. Advances in the automation of a very sensitive mouse sperm assay

The preliminary data on the effects of low x-ray doses on sperm head dimensions were presented in the last progress reports. Subsequently the data have been subjected to further statistical analyses resulting in further improvements in the sensitivity of this quantitative approach. The following is an abstract submitted and accepted for presentation at the 1979 annual meeting of the American Statistical Association and the North American Regions of the Biometric Society to be held in August 1979 at Washington, D.C.

Abstract: Mahalanobis distance and linear regression to optimize evaluation of dose response. The problem is to classify an object as "normal" or "abnormal" based on a vector of measurements. Initially the objects are unclassified, but for each there is available an independent, discrete measurement which is related to the likelihood that the object is abnormal. This situation occurs in studying the effects of x-irradiation on sperm morphology. An automated image analysis system makes several measurements on each sperm. It is not known *a priori* which sperm are normal and which abnormal, but it is expected that increased doses lead to increased frequency of abnormal sperm.

We describe a method where the Mahalanobian distance of each object from the mean of a control (0 dose) group is used to classify it as normal or abnormal. By regressing either Mahalanobian distances or precent classified abnormal on dose the resulting dose response curve can be used to find the most sensitive subset of variables.

B. Detecting and quantitating the effects of testicular toxins with flow cytometry.

In collaboration with the LLL team developing the flow systems instrumentation to study the effects of testicular toxins on sperm, the following paper was presented at the 10th annual meeting of the Environmental Mutagen Society in spring 1979.

Abstract: Flow cytometric evidence for radiation induced DNA variability in mature mouse sperm. Mutagenic exposure results in production of morphologically abnormal sperm and may cause variability in sperm DNA content. Flow cytometric measurements of epididymal sperm taken from $B6C3F_1$

mice 5 weeks after local x-irradiation to the testes show a dose-dependent fraction of cells with altered DNA stain content. Acriflavine-Feulgen stained sperm nuclei are measured in a flow cytometer that hydrodynamically orients them to control optical effects caused by their flat shape and high index of refraction. The fluorescent stain is excited by a laser beam orthogonal to the sample flow axis and flat surface of the nuclei. Fluorescence is detected both in line with (forward detector) and orthogonal to the laser beam. Fluorescence histograms from the forward detector are symmetric and are fit well by the sum of two normal distributions with the same mean, one with a coefficient of variation (CV) of about 3% and the other with a CV of about 8%. These CV's are independent of dose. The proportion of cells in the broad component is 0%, 30%, and 50% at doses of 0, 300, and 600 rads respectively. Symmetry of the histograms from the forward detector and correlated changes in signals from the two detectors indicate that these changes in fluorescence are not caused by radiation induced shape changes in the sperm. The interpretation of fluorescence variations as DNA content variations is tentative because we do not resolve the expected difference between the X and Y bearing sperm in these samples. This difference is seen in bull sperm nuclei.

Futher studies are in progress to define in more detail the dose response characteristics of this phenomena using two different strains of mice.

II. Work in Progress:

A. Comparative study of sperm production in $B6C3F_1$ mice from different suppliers.

The hybrid males, (C57BL/6 x C3H) F_1 or $B6C3F_1$ have repeated shown themselves to be a very useful genotype for the mouse sperm abnormality

assay. The increasing national popularity of this bioassay has seriously reduced the availability of these mice. We have identified five major suppliers of mice that produce $B6C3F_1$ males: a) The Jackson Laboratory, Bar Harbor, Maine; b) Simonsen Laboratories Inc., Gilroy, Ca; c) Charles River Breeding Laboratory, Wilmington, Mass; d) Timco Breeding Laboratories, Houston, Texas; e) Biobreeding Laboratories, Ottowa, Canada.

However since these have their own different sources of parental stocks for this hybrid, we could only speculate that the hybrids from the different suppliers will in fact show the same spontaneous values of sperm abnormalities and the same senstivities to unknown agents. We therefore propose to study the mice from each supplier by comparing the spontaneous levels, response to ionizing radiation and response to the hydrocarbon benzo(a)pyrene.

The findings of these comparions will be useful for all persons using the mouse sperm assay.

B. Studies with coal gasification and oil shale products and effluents.

The purpose of these studies is to determine the efficiency of the mouse sperm test for complex environmental mixtures from energy technologies.

Coal Gasification Effluents: In the fall of 1977, the Lawrence Livermore Laboratory successfully completed Coal Gasification Experiment No. 2 at Hoe Creek, Wyoming. One hundred gallons of water were sampled shortly after the burn from a well close to the burn site. A preliminary chemical analyses of this water showed moderate to high levels of pollutants especially of organic carbons and phenols. Although our role in this project was to determine whether this water was active in the mouse sperm test, there was insufficient final material to properly assess these effects.

There is now a new underground coal gasification experiment underway at Hoe Creek with the burn scheduled for August 1979. A number of wells have

been drilled and water will be sampled before and after the burn at various distances from the burn site. It will again be our role to determine whether this water is active in the mouse sperm test and to assess any changes in this activity with distance from the burn site.

Since hydrocarbons are expected to be the pollutant of major biological importance, we developed our study to first test the lipid soluble fraction of water by injecting various concentrations into mice using corn oil as a vehicle. The lethal dose (LD50, subsequently referred to as X) of the extracted fraction is determined in a conventional manner. Then 5 groups of 3 mice each (genotype $B6C3F_1$) will receive doses of 0.5X, 0.3 (1/2X), 0.03 (1/2X) and 0 (1/2X), administered in 5 consecutive, daily, intraperitoneal injections. Mice are to be sacrificed four weeks later: sperm morphology will be scored and dose response curves will be generated. Results are to be expressed as a ratio of the dose required to double background values to the LD50. This ratio will be compared to results obtained with 60 standard chemical agents (Wyrobek and Bruce, The induction of sperm abnormalities in mice and humans, in Chemical Mutagens: Principles and Methods for their detection., ed. A. Hollander, Plenum Press, New York, 1978).

Oil Shale Products and Effluents: In the Biomedical Sciences Division of LLL we have recently initiated a multi-testsystems approach to assessing the cytotoxicity and mutagenicity of oil shale products and effluents. There has been some suggestion that oil shale is mutagenic on contact with skin.

The U.S. Navy recently purchased 100,000 barrels of oil extract from oil shale by an above ground retort process by the Paraho Development Corp. in Rifle, Colorado. This oil is high in nitrogen and contains aromatic compounds. The Navy has hydrogenated a portion of this oil to reduce the content of nitrogen and aromatic compounds. Our role in the LLL project is to

assess the health impact of this oil and to determine whether the unhydrogenated Parahoe oil differs from the hydrogenated oil in the ability to induce germ cell toxicity and sperm abnormalities in mice. We propose to begin our analyses of these oils by injecting various concentrations of the oils into mice using corn oil as a vehicle. The LD50 and dose response curves will be obtained as described for the coal gasification effluents, above. For each oil, results will be expressed as a ratio of the dose required to double background values to the LD50. This ratio will be compared to that obtained with other oils and to those obtained with 60 standard agents.

Automated Mouse Sperm assay: The automated scoring of mouse sperm morphology requires high speed, automatic scanning of sperm images under the microscope, search capabilities, contour identification, and shape analyses. In collaboration with I.T. Young of the Biomedical Sciences Division at LLL, a scanning light microscope has been used to visualize images of sperm head shape which are then transfered to a PDP-11 computer. We have successfully transfered many such images using the ACUITY system (U.S. DOE/LLL/EV-81-75 "Quantitative Microscopy"). The programs necessary to research for sperm and to identify their contours are already available. We are in the process of developing the programs to analyze the shape of the sperm head. Our goal is to complete the entire process from scanning to analyses of shape at the rate of about 2 seconds per sperm.

RELATIONSHIP TO OTHER PROGRAMS:

Collaboration with other programs within and outside the Division continues to be very strong and productive. The following is a list of collaborators, their programs and the topics of collaboration:

A. Within the Biomedical Division

1. With the funding of B.L. Gledhill's program, "Sperm as Indicators of Hazard" DOE/ASEV RPIS 000254, we have studied the inheritance of sperm abnormalities in F_0, F_1, F_2 and backcross generations for 4 strains of mice and we have studied the genetic inheritance of sperm abnormalities in mice carrying reciprocal translocations.
2. In conjunction with B.L. Gledhill and the above program as well as the DOE programs "Flow Systems Application" DOE/ASEV RPIS 000257, and "Flow Systems Development" DOE/ASEV RPIS 000256, we have made use of available state of the art flow technology to automate the quantitation of abnormal sperm.
3. In collaboration with R. Balhorn, "DNA, Chromatin and Chromosomes" DOE/ASEV RPIS 000266 and with the funding of the program "Sperm as Indicators of Hazard" we have found that the sperm nucleoproteins is made up of at least two moieties (see publication No. 6). We are now looking at the abnormal sperm of mutagenized mice to determine whether they differ from the normal in nucleoprotein composition.
4. Fred Hatch, "Toxicology of Coal Gasification" DOE/ASEV RPIS 002631, is coordinating the testing of effluents from <u>in situ</u> coal gasification technologies.
5. Jim Felton and L. Dobson, "Radiosensitivity of Oocytes" DOE/ASEV RPIS 000252 and "The Mouse System as Monitor of Chemical Toxicity" EPA-IAG-D6-E681-AQ, have supplied us with males exposed <u>in utero</u> to x-rays and methylcholanthrene. Together with J. Felton we have also carried out together much of the work reported here on the genetics of AHH induction in relation to the production of abnormal sperm.

6. B. Mayall, "Health Effects - Fossil Fuels" DOE/ASEV RPIS 002629, is coordinating a broad based study of health effects in coal and petrochemical workers. We are planning to evaluate their semen.

B. Outside the Division

1. We have accepted an invitation to participate in Phase II of the ICI/MRC/NIEHS collaborative study on carcinogen/noncarcinogen paired compounds coordinated by Dr. J. Ashby, Imperial Chemical Industries, Ltd., U.K. and Dr. F. de Serres of NIEHS.

2. We have been asked by numerous laboratories, most within the USA, to help them score animals for morphologically abnormal sperm or to help them set up the mouse sperm assay. For example:

   a) Drs. Mahlum and Sikov, Battelle N.W. Laboratory, Richland, Washington to score rat sperm from animals exposed to electromagnetic radiation and plutonium.

   b) Dr. Arthur Penn, New York University Medical Center, New York to score sperm of roosters exposed to methylcholanthrene.

   c) Dr. Rainer Lange of Schering Company, Berlin, Germany to score standard sperm to help him set up the sperm assay at his company.

   d) Dr. J. Topham of Imperial Chemical Industries Ltd., U.K. to help him set up the sperm assay at his company.

   e) Dr. D. Brusick and M. Bakshi of Litton Biometrics, Ltd., Maryland to set up the mouse sperm assay at Litton.

   f) Dr. E. Wei of School of Public Health in Berkeley, to help him set up the mouse sperm assay.

   g) Dr. D. Perrin of the Pasteur Institute in Paris to help that institute initiate a commercially available mouse sperm assay.

3. With W.R. Bruce and A. Hugenholtz of the Ontario Cancer Institute, Canada, we have collaborated on studies of the heritibility of sperm abnormalities have have recently published an extensive review of the field of abnormal sperm in mice and humans (see publication No. 3).

4. We have numerous collaborations to analyze the semen of humans occupationally or therapeutically exposed to potentially hazardous agents.

   a) Dr. D. Whorton, Director of Labor Occupational Health Program, U.C. Berkeley, is supplying us with sperm of workers exposed to pesticides.

   b) Drs. Ellis Cohen and Jay Brodsky, Dept. of Anesthesia, Stanford Medical School, are supplying us with sperm from anesthesiologisits.

   c) Dr. M.L. Meistrich, Dr. E. Steinberger in conjunction with the heads of 4 clinical departments of the M.D. Anderson Hospital and Tumor Institute will supply us with samples of sperm from patients on chemotherapy.

   d) Dr. Ed Schneider of the National Institute of Aging, Maryland, has sent us the semen of young and old human males so that we can study the effects of age on semen quality.

Publications on project to date:

PUBLICATIONS:

1. Wyrobek, A.J. "Sperm Shape Abnormalities in the Mouse as an Indicator of Mutagenic Damage," in The Testis in Normal and Infertile Men, P. Troen and H.R. Nankin, Eds., (Raven Press, New York, 1977), pp. 519-523.

2. Wyrobek, A.J. "Changes in Mammalian Sperm Morphology After X-ray and Chemical Exposures," Genetics Suppl. (in press) May 1979.

3. Wyrobek, A.J. and Bruce, W.R., "The Induction of Sperm Shape Abnormalities in Mice and Humans," in Chemical Mutagens: Principles and Methods for Their Detection, A.H. Hollander and F.J. deSerres, Eds., (Plenum Press, New York, 1978), Vol. 5, 257-285.

4. Wyrobek, A.J. and Gledhill, B.L., "The Sperm Test: A Short-Term, In Vivo, Mammalian Bioassay for Agents Hazardous to the Male Germ Cells," Proc. Symp. Applicability of Short-Term Bioassays in the Fractionation and Analysis Complex Environmental Mixtures, abst. p. 53 (1978).

5. Wyrobek, A.J., Watchmaker, G., Foote, J. and Singh, H., "The Effects of Methyl Methanesulfonate and Dimethylnitrosamine on Sperm Production in the Syrian Hamsters," in Developmental Toxicology of Energy-Related Pollutants, Malhum, D. and Sikov, M., Eds. (DOE, Oak Ridge, 1978), pp 68.

6. Balhorn, R., Weston, S. and Wyrobek, A.J., "Asynchrony in the Synthesis of Murine Protamines," Fed. Proc. 37, 2038.(1978)

EPA DOCUMENTS:

1. Wyrobek, A.J. "Morphology variants in damaged sperm," EPA-1AG-D6-E681-AN Progress Report for July 1975 to December 1975.

2. Wyrobek, A.J. "Morphology variants in damaged sperm," EPA-1AG-D6-E681-AN Progress Report for January 1976 to June 1976.

3. Wyrobek, A.J. "Morphology variants in damaged sperm," EPA-1AG-D6-E681-AN Progress Report for July 1976 to December 1976.

4. Wyrobek, A.J. "Morphology variants in damaged sperm," EPA-1AG-D6-E681-AN Progress Report for January 1977 to June 1977.

5. Wyrobek, A.J. "Morphology variants in damaged sperm," EPA-1AG-D6-E681-AN Progress Report for July 1977 to December 1977.

6. Wyrobek, A.J., "Morphological Variants in damaged sperm," EPA-1AG-D5-E681-AN, Comprehensive summary three-year progress report for July 1975 to July 1978 and "Sperm shape abnormalities induced by energy-related hydrocarbons and industrial chemicals," Proposal for renewal.

7. Wyrobek, A.J., "Sperm shape abnormalities induced by energy-related hydrocarbons and industiral chemicals," EPA-1AG-D6-E681-AN Progress Report for July 1978 to December 1978.

8. Wyrobek, A.J., Watchmaker, G., Foote, J. and Singh, H., "Effects of methyl methanesulfonate and dimethylnitrosamine on sperm production in Syrian hamster," EPA Technical Report (submitted).

9. Wyrobek, A.J., Watchmaker, G. and Gledhill, B.L., "Strain variations in radiosensitivity of germinal tissue in male mice," EPA Technical Report (submitted).

10. Wyrobek, A.J., and Gledhill, B.L., "Monitoring human semen in the workplace," EPA Technical Report (submitted).

11. Wyrobek, A.J., and Bruce, W.R., "Sperm-shape abnormalities in mice and humans," EPA Technical Report (submitted).