LA-UR-81-986

CONF-810357--1

MASTER

TITLE: GENOTOXIC EFFECTS OF SUNLIGHT-ACTIVATED WASTE WATERS

AUTHOR(S): Gary F. Strniste, LS-3
David J. Chen, LS-3
Richard T. Okinaka, LS-3

SUBMITTED TO: PROCEEDINGS OF THE FDA'S SEVENTH SCIENCE SYMPOSIUM ON "PHOTOCHEMICAL TOXICITY"

DISCLAIMER

This book was prepared as an account of work sponsored by an agency of the United States Government. Neither the United States Government nor any agency thereof, 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 any agency thereof. The views and opinions of authors expressed herein do not necessarily state or reflect those of the United States Government or any agency thereof.

By acceptance of this article, the publisher recognizes that the U.S. Government retains a nonexclusive, royalty-free license to publish or reproduce the published form of this contribution, or to allow others to do so, for U.S. Government purposes.

The Los Alamos Scientific Laboratory requests that the publisher identify this article as work performed under the auspices of the U.S. Department of Energy.

University of California

LOS ALAMOS SCIENTIFIC LABORATORY
Post Office Box 1663 Los Alamos, New Mexico 87545
An Affirmative Action/Equal Opportunity Employer

Form No. 836 R3
St. No. 2629
12/78

UNITED STATES
DEPARTMENT OF ENERGY
CONTRACT W-7405-ENG. 36

DISTRIBUTION OF THIS DOCUMENT IS UNLIMITED

Genotoxic Effects of Sunlight-Activated Waste Water

Gary F. Strniste, David J. Chen and Richard T. Okinaka

Genetics Group, Life Sciences Division

Los Alamos National Laboratory

Los Alamos, NM 87545

Running Title: Photoactivated Waste Water

Key words (phrases):

near ultraviolet light

sunlight

shale oil retort process water

Chinese hamster ovary cells

human skin fibroblasts

mutagenicity

cytotoxicity

INTRODUCTION

As natural petroleum reserves are consumed, it becomes more apparent that the U.S. will have to supplement its liquid energy supplies with synthetic fuels to meet projected energy demands. Of great promise are the organic reserves locked in vast oil shale deposits located primarily in the western U.S. Estimates of hundreds of billions of barrels of economically recoverable crude oil have been made from surveys of oil shale deposits located in Colorado, Utah and Wyoming (19). However, the processes involved in the mining, retorting, upgrading and refining of oil shale will be accompanied by potential environmental and health-related risks.

One major problem associated with the development of an oil shale industry in this semi-arid region will be the utilization of vast quantities of water and the production of process waters, a by-product of retorting which may amount to as much as 3 barrels for every barrel of crude oil produced. Due to its intimate contact with the oil during retorting, process waters, after separation in holding tanks, contain substantial amounts of aqueous-soluble organic materials. These dissolved components most likely contribute to the genotoxic properties of these waters when assessed in the Ames/Salmonella bioassay (5).

For several years, my laboratory has been investigating the process of photoactivation by near ultraviolet light (NUV) of certain classes of promutagenic/procarcinogenic compounds in an attempt to relate DNA structural damage induced *in vitro* to the genotoxic effects witnessed in cultured mammalian cell assays (4, 11, 13, 15). As an extension of this work, we have applied this "photoactivation process" to assess the genotoxic potential existing in by-products of shale oil (1, 5, 12, 14,

16). This report will consider a particular retort process water and its cytotoxicity and mutagenicity in cultured rodent cells or human skin fibroblasts after activation by NUV or natural sunlight.

METHODS

Test Materials. We have assessed for genotoxic potential process waters obtained from three retort processes (above ground, modified *in situ*, and true *in situ*) utilizing oil shale from deposits located in the Green river formation in the western U.S. Although all exhibit photo-induced genotoxic properties (5, 12, 14), this report will concern only the most active water, which was obtained from a holding tank for shale oil produced by an above-ground retort. This process water was filtered through Whatman #42 paper and a Millipore 0.2μ Swinnex unit before use.

Cell cultures. Chinese hamster ovary cells (line AA8-4) were obtained from Dr. L. Thompson (Lawrence Livermore National Laboratory). This cell line, heterozygous at the adenine phosphoribosyl transferase locus (17), was cultured under conditions described elsewhere (14). Fig. 1 outlines the basic scheme for determining the cytotoxic and mutagenic events witnessed in CHO cells. The genetic marker examined in this report is the X-linked, recessive hypoxanthine-guanine phosphoribosyl transferase (HGPRT) locus. This mutation assay system has been described elsewhere (6, 7, 14). Selection for $HGPRT^-$ mutants is accomplished by growth of treated cell populations in medium containing 10 μM of the toxic base analog 6-thioguanine, after a maximal expression period of 8 days incubation post-treatment (3, 14). Plating efficiencies of non-treated CHO cells are 90-100%.

Cultured human skin fibroblasts were purchased from the American Type Culture Collection (Rockville, MD). Normal (NF) cells (CRL 1295) and xeroderma pigmentosum (XP) cells (CRL 1223 or XP12BE) were grown in Dulbecco's modified Eagle's medium (DMEM) containing 20% (v/v) fetal calf

serum (13). A similar experimental protocol as diagramed in Fig. 1 for CHO cells was used to determine photo-induced genotoxicity in human cells with the following modifications: (1) for toxicity measurements between 100 and 1000 cells were plated, for mutagenicity measurements 3-6 plates at 100K cells/plate were used for each dose point; (2) the cell attachment period was 20-22 hr; (3) for toxicity measurements treated plates were incubated a total of 8-11 days with an intermittent feeding on the 5th day; (4) maximal expression of $HGPRT^-$ mutants was obtained in 6-8 days (a minimum of 4 cell divisions); and, (5) for selection of $HGPRT^-$ mutants cells were plated at 25K/dish (35-40 dishes per dose point) in medium with 10% fetal calf serum plus 30 μM 6-thioguanine and incubated for 14 days with an intermittent feeding on the 7th day. Corresponding efficiency of plating (E.O.P.) determinations were performed for each dose point. The E.O.P. for non-treated NF cells was 50-75%, for XP cells was 25-40%.

Irradiation. The source of FUV (primarily 254 nm wavelength) was a single 15 watt germicidal lamp (General Electric, #G15T8). The incident fluence was 0.5 J per m^2 per sec. The source of NUV (300-400 nm wavelength) was 2 parallel 15 watt black lights (General Electric, #F15T8 BLB). The incident fluence averaged 6.5 J per m^2 per sec. Calibration of these sources is described elsewhere (13, 14). For experiments utilizing natural sunlight, plates containing attached cells were exposed for various times on the rooftop of the Health Research Laboratory in Los Alamos, NM (lat. 35°-53'-11" N, long. 106°-18'-58" W at an elevation of 7450 ft above sea level). Sunlight experiments reported here were performed from 12:30 pm to 1:30 pm during Dec. 1980 and Jan. 1981; the total measured fluence (200-2500 nm wavelength) was 850-950 J per m^2 per sec.

RESULTS AND DISCUSSION

Pre-treatment of CHO cells with shale oil retort process waters and subsequent exposure to NUV resulted in both cytotoxic and mutagenic responses (5, 12, 14, 16). Mutation frequencies induced at the HGPRT locus were as great as 50% that witnessed for the potent skin carcinogen, FUV (14, 16) at equivalent surviving fraction of cells. However, the magnitude of this genotoxic response was dependent on the process water used; in situ retort process waters were less photoactive than a corresponding above-ground retort process water. These observations suggested that natural sunlight, which has a NUV component, may have a similar effect. To test this hypothesis, CHO cells pre-treated with above-ground retort process water (1:270 dilution for 1 hr in the dark) were exposed for various times to natural sunlight. The survival data (in terms of colony forming ability) are shown in Fig. 2. The exposure time required to reduce cell survival to 37% (D_{37}) is ~50 seconds, whereas non-treated CHO cells are inactivated to 37% surviving fraction only after ~6 minutes exposure to sunlight. This represents a 7-fold increase in the photosensitivity of CHO cells pre-treated with process water compared to untreated cells. The estimated total solar irradiance (200-2500 nm) in Los Alamos, NM, during December, 1980 and January, 1981 averaged ~900 J per m^2 per second. Approximately 3% of the solar spectrum is in the NUV (300-400 nm wavelength). Therefore, the fluence of sunlight necessary to reduce survival to 37% for process water-treated CHO is 4.5×10^4 J per m^2 of which ~1400 J per m^2 is NUV. This NUV fluence is the same as that obtained in experiments using NUV generated by black lights (14) indicating the role of this component of the solar spectrum in the photoactivation process.

In Fig. 3 the numbers of HGPRT$^-$ mutants (cells resistant to

6-thioguanine) induced by exposure of CHO cells to process water plus sunlight or to sunlight only are plotted versus the percent surviving fraction of cells. At the D_{37} for colony forming ability, there is a 9-fold increase in the number of photo-induced mutants in CHO cells pre-treated with process water compared to untreated cells after subtraction of the natural background mutation frequency (averaged ~1 per 10^5 cells). As noted for photo-induced cytotoxicity, the frequencies of photo-induced mutations in process water-treated CHO cells are the same for equivalent fluences of sunlight or black light NUV.

The potential environmental and health-related risks resulting from concomitant exposure to shale oil by-products (including process waters) and natural or artificial light are unknown. However, a primary target in human exposure will be the skin. The following experiments were designed incorporating cultured human skin fibroblasts as targets in order to assess better the photo-induced genotoxic properties (towards humans) of shale oil retort process waters. As in the CHO cell studies discussed above, we utilized one process water obtained from an above-ground retort facility. For comparative purposes, analogous cytotoxic and mutagenic studies were performed using FUV.

In Fig. 4 the survival (colony forming ability) of normal human skin fibroblasts (NF) is shown after pre-treatment with process water and subsequent NUV irradiation or exposure to FUV only. Due to the extreme cytotoxic properties of this water towards fibroblasts, a dilution of 1:700 of process water was necessary to effect a concentration resulting in no cytotoxicity (1 h exposure in the dark). At this dilution, a fluence of NUV of 1700 J per m^2 is necessary to reduce survival to 37% of control, untreated cultures. Treatment of NF cells with process water only (1 hr in

the dark) or with NUV only (fluences up to 3000 J per m^2) resulted in no cytotoxicity or mutagenicity.

In Fig. 5, FUV or process water plus NUV induced $HGPRT^-$ mutants in NF cells are plotted versus radiation fluence. In these experiments, the average background mutation rate (0.3 per 10^5 cells) has been subtracted from each data point. The establishment of conditions maximizing the response of this human cell/HGPRT mutation assay has been determined with embryonic human skin fibroblasts (1, 2) and those protocols were used in the experiments reported here.

The data in Figs. 4 and 5 are replotted in Fig. 6 in order to show $HGPRT^-$ mutants induced as a function of surviving fraction of cells. These data indicate that the frequency of induction of mutants (per surviving fraction of cells) at the HGPRT locus is the same for either FUV or process water plus NUV. At 37% surviving fraction of cells, the number of mutants induced by above-ground retort process water and NUV in human skin fibroblasts is about 60% that observed in CHO cells, and the number of FUV-induced mutants in NF cells is only about 20% that observed for CHO cells exposed to FUV (14). These differences in mutation frequencies observed can be accounted for, at least in part, by differences in repair capabilities in rodent versus human cells (8, 18).

In recent studies, we have examined the cytotoxic effects induced in repair deficient human skin fibroblasts by process water and NUV. Xeroderma pigmentosum (XP) cells obtained from a patient of complementation group A were used in this study. These cells are extremely sensitive to FUV and certain classes of chemical compounds due to deficiencies in their excision repair capacity (9, 10). In Fig. 7 the survival (colony forming ability) of NF and XP cells pre-treated with process water (1:700 dilution)

and subsequent exposure to NUV is shown. The insert figure shows the survival of these two cell types exposed to FUV radiation. In both examples, XP cells show an acute cytotoxic response compared to NF cells (5-fold and 16-fold increase in sensitivity when comparing D_{37} values of process water plus NUV treatment or FUV treatment, respectively). This result suggests that the magnitude of genotoxicity of photoactivated process water in human cells is influenced by repair capabilities existing in the cell. The decrease in the magnitude of sensitivity seen for XP cells (compared to NF cells) treated with process water plus NUV versus FUV radiation could possibly result from a contribution to cell toxicity by non-DNA damage (e.g., membrane damage). We have observed a similar reduction in sensitivity in XP cells (compared to NF cells) for cytotoxicity induced by photo-activated polycyclic aromatic hydrocarbons (7). The role of repair in the modulation of mutagenic potential induced in human cells is the subject of current investigation.

CONCLUSIONS

1. Natural sunlight induces a genotoxic response in cultured CHO cells pre-treated with shale oil retort process water.
2. The NUV component of the solar spectrum is the apparent radiation responsible for photoactivition.
3. Cultured human skin fibroblasts are acutely sensitive to the genotoxic effects of photoactivated process water. The mutagenic potential of photoactivated process water in human cells is the same as that witnessed for an equivalent killing dose of the potent skin carcinogen FUV.
4. DNA repair processes are involved in modulating genotoxic effects of this photo-induced process.
5. The exact magnitude of the potential health-related and environmental risks resulting from photoactivation of retort process waters and other oil shale by-products is unassessed at this time. Our demonstration that a significant rate of mutation occurs in cultured human cells exposed to high dilutions of process waters and fluences of NUV comparable to that encountered during nominal exposure to sunlight suggests that such assessment is a prerequisite to minimal risk development of our oil shale resources.

ACKNOWLEDGMENTS

The technical assistance of Ms. J. Nickols, Ms. E. Wilmoth and Mr. E. Martinez is greatfully acknowledged. This work was performed under the auspices of the United States Department of Energy.

REFERENCES

1. Chen, D.J. Okinaka, R.T. and Barnhart, B.J. (1980) Quantitation of Mutations induced by metabolically activated mutagens in normal human fibroblasts. Environ. Mutagenesis 2, 294.

2. Chen, D.J., Okinaka, R.T., Strniste, G.F. and Barnhart, B.J. (1981). Induction of 6-thioguanine resistant mutations by rat liver homogenate (S9)-activated promutagens in human embryonic skin fibroblasts (manuscript submitted).

3. Chen, D.J. and Strniste, G.F. (1981) Induction of mutations at five genetic loci in CHO cells by near ultraviolet light-activated shale oil process water. Environ. Mutagenesis (in press).

4. Hoard, D.E., Ratliff, R.L., Bingham, J.M. and Strniste, G.F. (1981) Reactions induced in vitro between model DNA and benzo(a)pyrene by near ultraviolet light. Chem-Biol. Inter. 33, 179-194.

5. Holland, L.M. and Stafford, C.G., compilers (1981) The Los Alamos integrated oil shale health and environmental program: A status report. Los Alamos National Laboratory Report LA-8665-SR, pp. 54-65.

6. O'Neill, J.P., Brimer, P.A., Machanoff, R., Hirsch, G.P. and Hsie, A.W. (1977). A quantitative assay of mutation induction at the hypoxanthine-guanine phosphoribosyl transferase locus in Chinese hamster ovary cells (CHO/HGPRT system): development and definition of the system. Mutat. Res. 45, 91-101.

7. O'Neill, J.P., Couch, D.B., Machanoff, R., San Sebastian, J.R., Brimer, P.A. and Hsie, A.W. (1977) A quantitative assay of mutation induction at the hypoxanthine-guanine phosphoribosyl transferase locus in Chinese hamster ovary cells (CHO/HGPRT system): utilization with a variety of mutagenic agents. Mutat. Res. 45, 103-109.

8. Painter, R.B. and Cleaver, J.E. (1969) Repair replication, unscheduled DNA synthesis and the repair of mammalian DNA. Radiat. Res. 37, 451-466.

9. Robbins, J.H., Kraemer, K.H., Lutzner, M.A., Festaff, B.W. and Coon, H.G. (1974). Xeroderma pigmentosum: An inherited disease with sun sensitivity, multiple cutaneous neoplasms and abnormal DNA repair. Ann. Intern. Med. 80, 221-248.

10. Setlow, R.B. (1978) Repair deficient human disorders and cancer. Nature 271, 713-717.

11. Strniste, G.F. and Brake, R.J. (1980) Photoactivity of near ultraviolet light-irradiated benzo(a)pyrene. Fed. Proc. 39, 2011.

12. Strniste, G.F. and Brake, R. J. (1980) Toxicity and mutagenicity of shale oil retort product waters photoactivated with near ultraviolet light. Environ. Mutagenesis 2, 268.

13. Strniste, G.F. and Brake, R.J. (1981) Cytotoxicity in human skin fibroblasts induced by photoactivated polycyclic aromatic hydrocarbons. In: Polynuclear Aromatic Hydrocarbons: Chemistry and Biological Effects, eds. A. J. Dennis and W. M. Cooke, Battelle Press, Columbus, OH (in press).

14. Strniste, G.F. and Chen, D.J. (1981) Cytotoxic and mutagenic properties of shale oil byproducts I. activation of retort process waters with near ultraviolet light. Environ. Mutagenesis 3 (in press).

15. Strniste, G.F., Martinez, A.M., Martinez, E. and Brake, R.J. (1980) Photo-induced reactions of benzo(a)pyrene with deoxyribonucleic acid in vitro. Cancer Res. 40, 245-252.

16. Strniste, G.F., Okinaka, R.T. and Chen, D.J. (1981) Photoactivation of shale oil by-products, Environ. Mutagenesis (in press).

17. Thompson, L.H., Fong, S. and Brookman, K. (1980) Validation of conditions for efficient detection of HPRT and APRT mutations in suspension-cultured Chinese hamster ovary cells. Mutat. Res. 74, 21-36.

18. Trosko, J.E. and Kasschau, M.R. (1967) Study of pyrimidine dimers in mammalian cells surviving low doses of ultraviolet radiation. Photochem. Photobiol. 6, 215-219.

19. Yen, T.F. and Chilingarian, G.V. (1976) Introduction to oil shales. In: Oil Shale, eds. T.F. Yen and G.V. Chilingarian, Elsevier Scientific Publishing Co., Amsterdam, pp. 1-12.

FIGURE LEGENDS

Fig. 1. Protocol used for the determination of genotoxic potential of light activated mixtures using the CHO/HGPRT mutation system. The selective agent used was 6-thioguanine at 10 μM. See Methods section for details.

Fig. 2. Loss of colony forming ability of CHO cells exposed to shale oil retort process water plus natural sunlight (closed symbols) or to natural sunlight only (open symbols). Different symbols represent results from independent experiments.

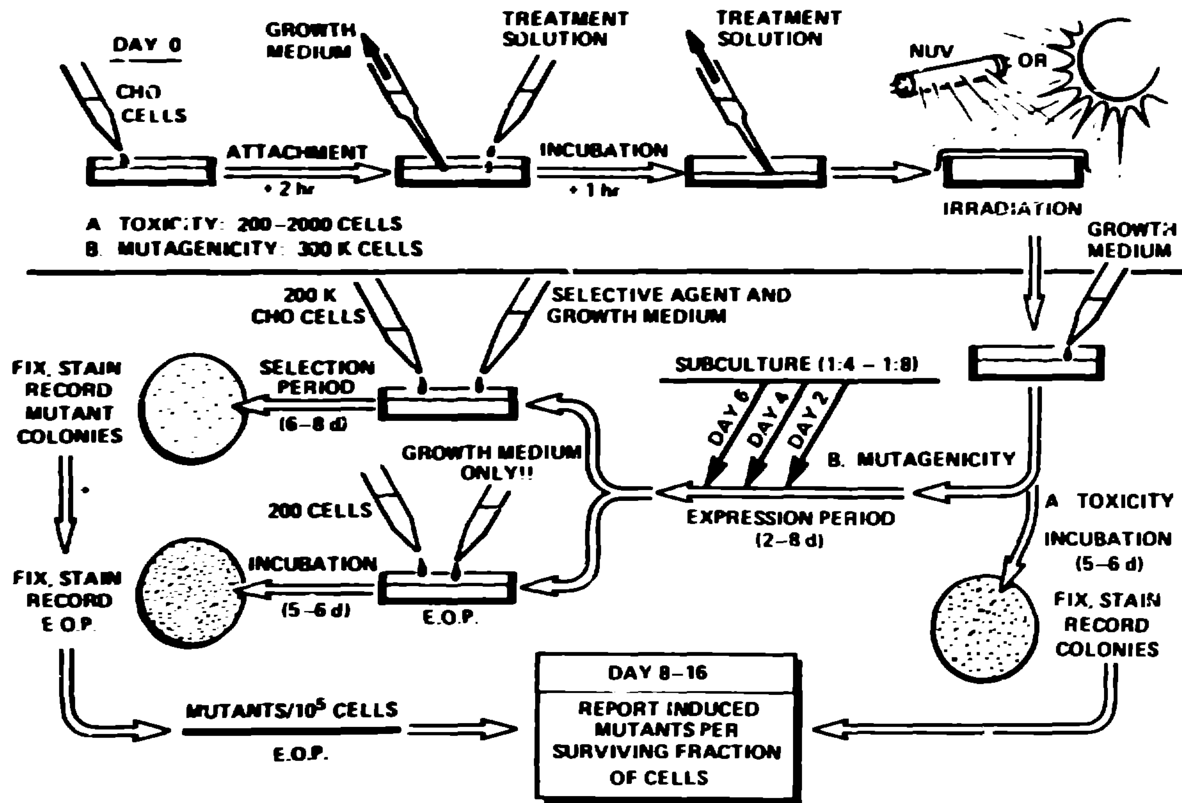
Fig. 3. 6-TG^R mutants ($HGPRT^-$) induced per surviving fraction of CHO cells after exposure to shale oil retort process water plus natural sunlight (—●—) or to sunlight only (—○—). Arrow bars represent ± one standard deviation.

Fig. 4. Loss of colony forming ability of cultured human skin fibroblasts exposed to shale oil retort process water plus NUV (open symbols) or to FUV only (closed symbols). Different symbols represent results from independent experiments.

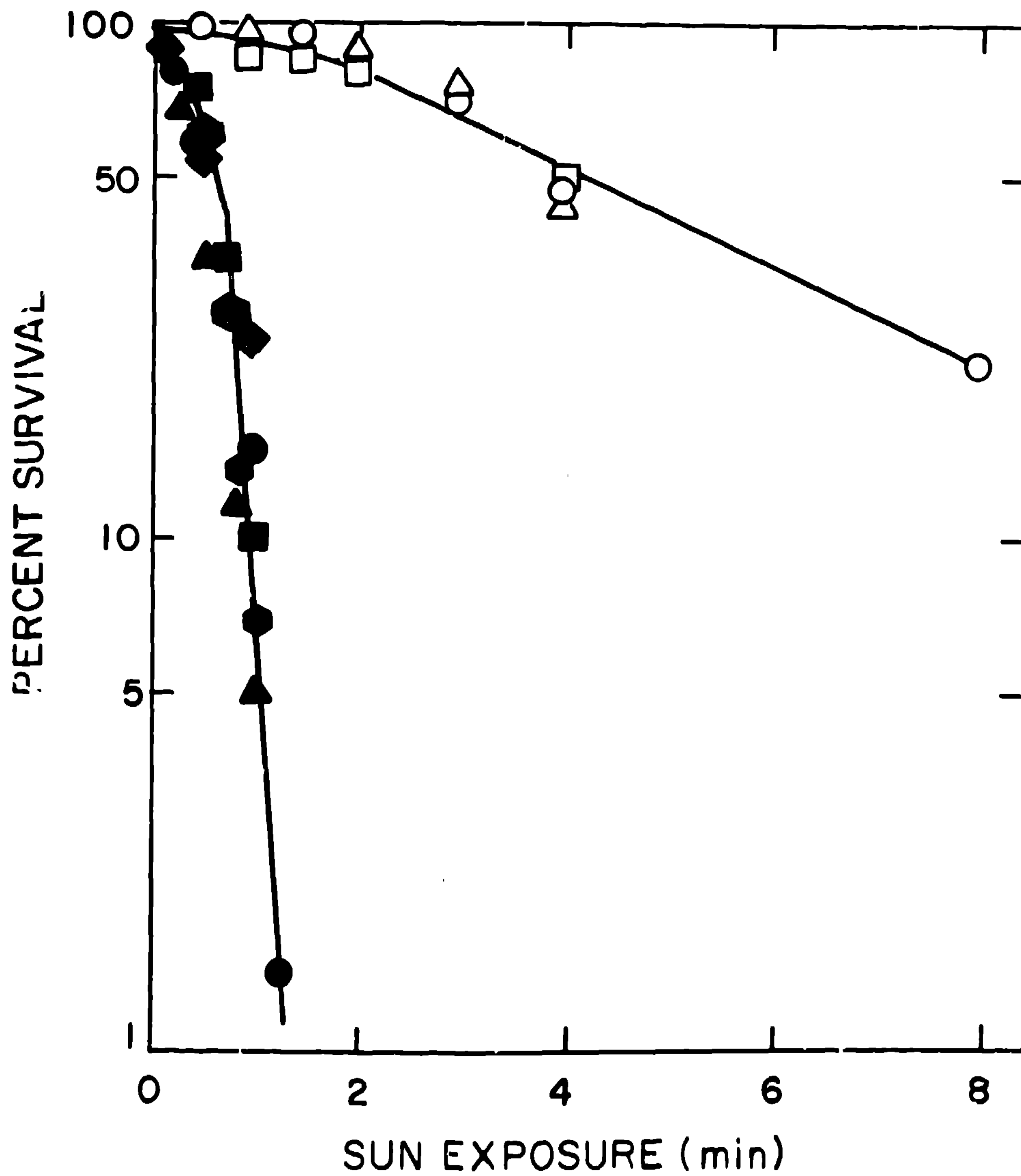
Fig. 5. 6-TG^R mutants ($HGPRT^-$) induced in cultured human skin fibroblasts exposed to FUV (upper curve) or shale oil retort process water plus NUV (lower curve).

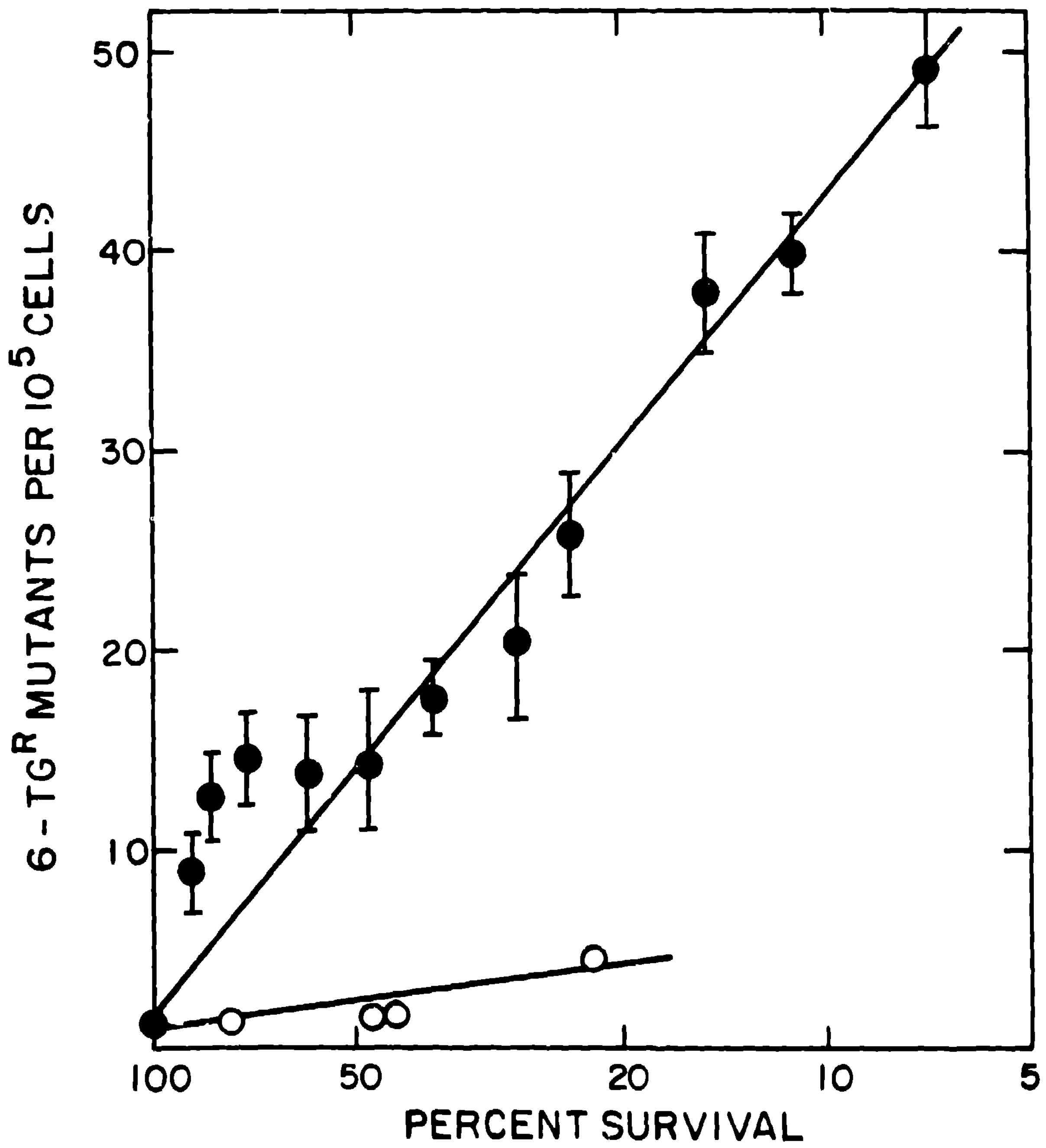
Fig. 6. 6-TG^R mutants ($HGPRT^-$) induced per surviving fraction of human skin fibroblasts after exposure to FUV (—▲—) or shale oil retort process water plus NUV (—●—).

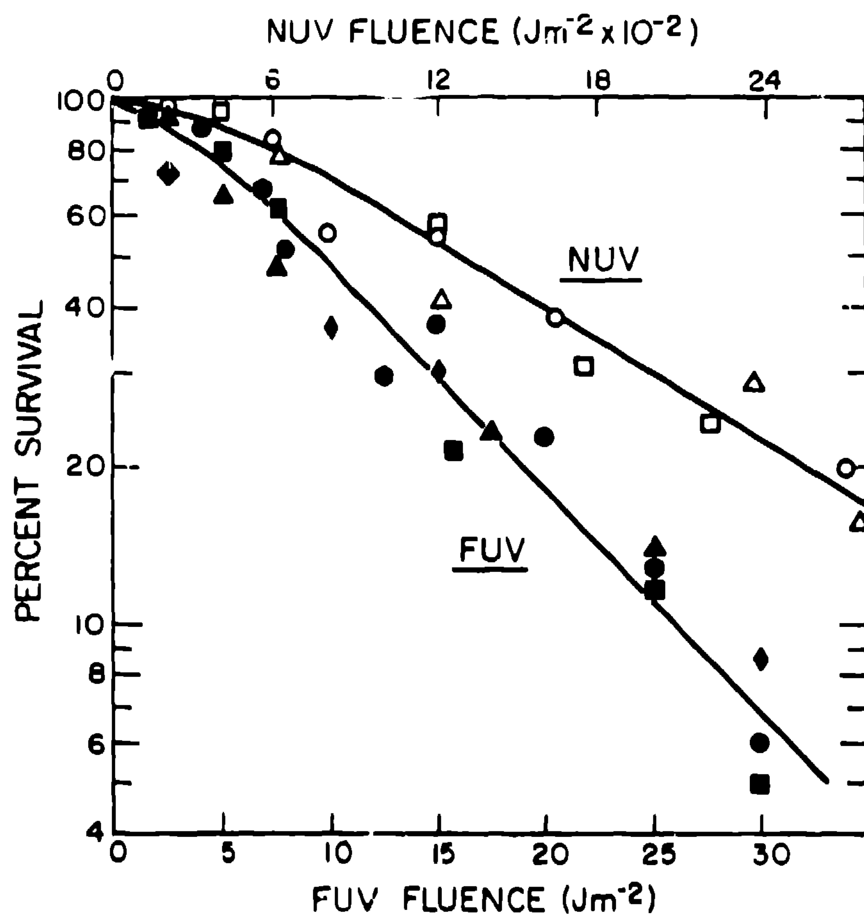
Fig. 7. Loss of colony forming ability in normal human skin fibroblasts (NF) and in xeroderma pigmentosum skin fibroblasts (XP) after exposure to shale oil retort process water plus NUV. Insert figure represents loss of colony forming ability in NF and XP cells after FUV exposure. Different symbols represent results from independent experiments.

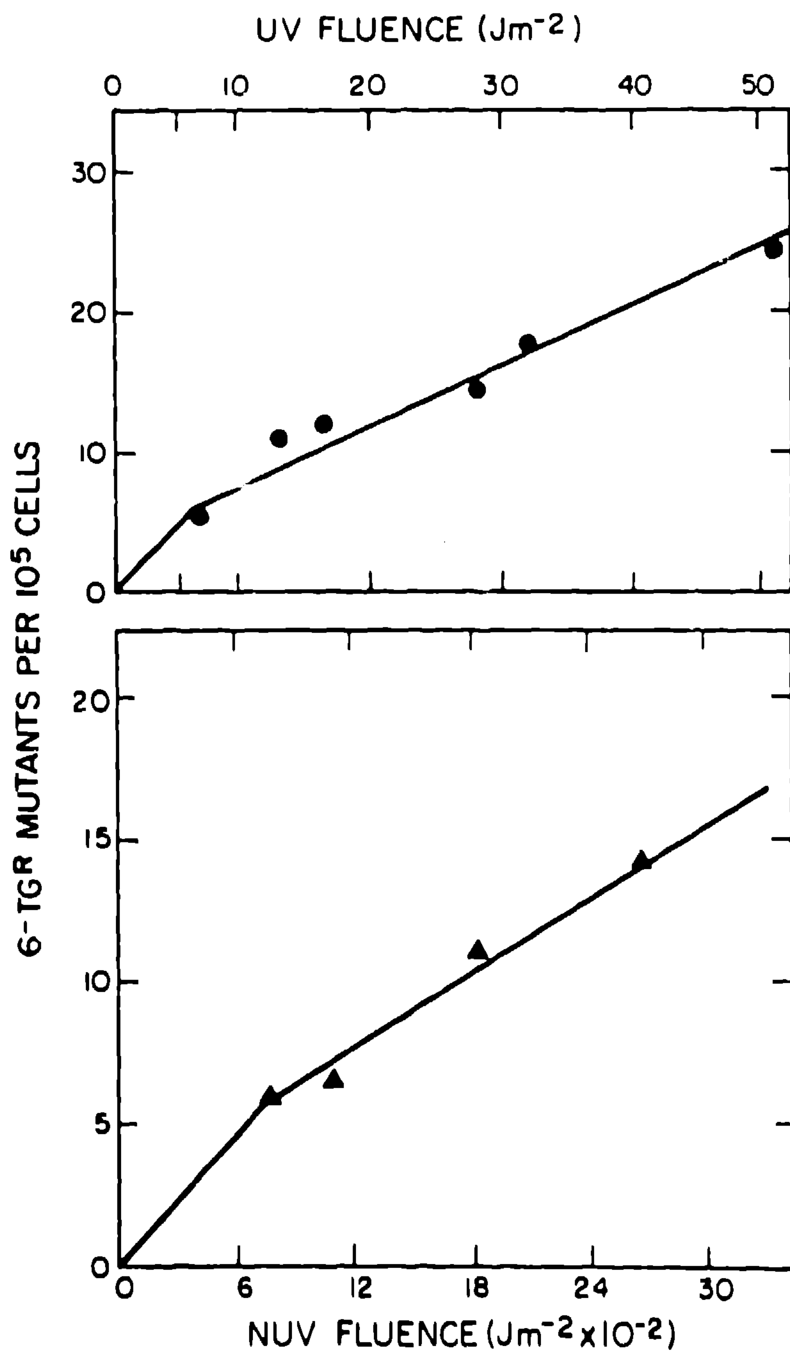


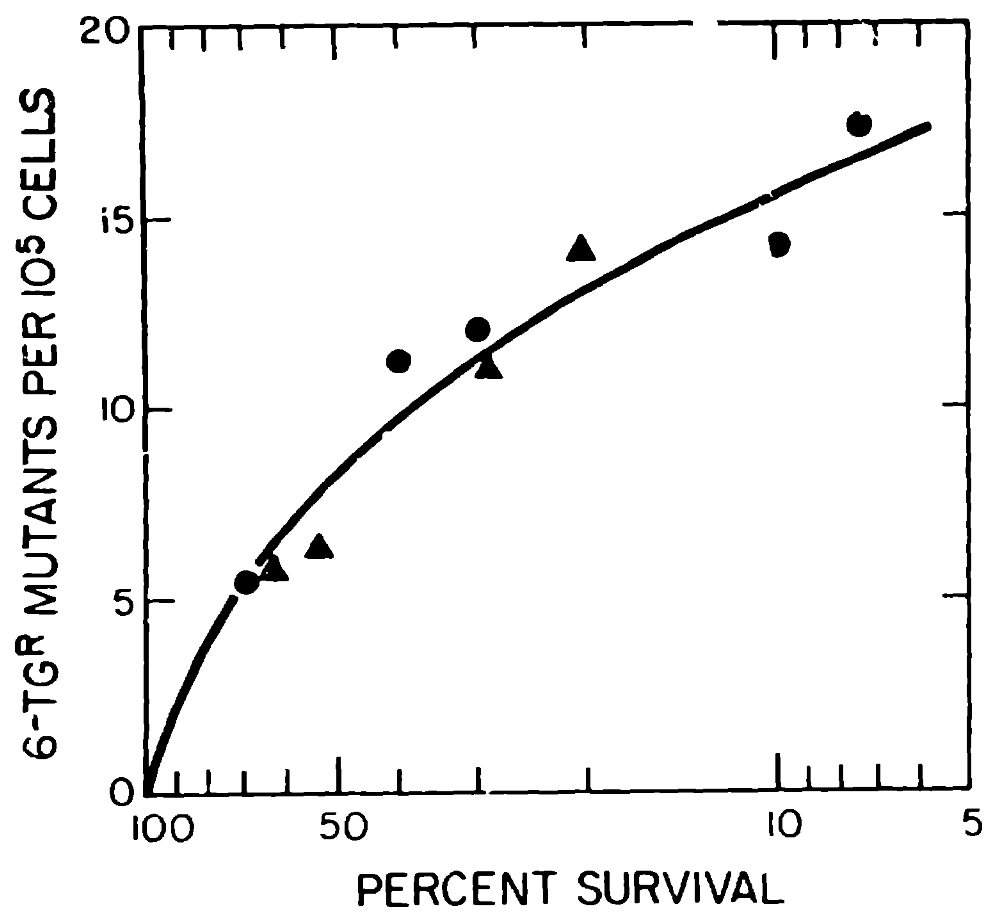
CHO CELL MUTATION ASSAY
DAY 0
CHO CELLS
GROWTH MEDIUM
TREATMENT SOLUTION
TREATMENT SOLUTION
NUV
OR
ATTACHMENT
+ 2 hr
INCUBATION
+ 1 hr
IRRADIATION
A TOXICITY: 200-2000 CELLS
B MUTAGENICITY: 300 K CELLS
GROWTH MEDIUM
200 K CHO CELLS
SELECTIVE AGENT AND GROWTH MEDIUM
FIX, STAIN RECORD MUTANT COLONIES
SELECTION PERIOD (6-8 d)
SUBCULTURE (1:4 - 1:8)
DAY 6
DAY 4
DAY 2
B. MUTAGENICITY
GROWTH MEDIUM ONLY!!
200 CELLS
EXPRESSION PERIOD (2-8 d)
A TOXICITY
INCUBATION (5-6 d)
FIX, STAIN RECORD E.O.P.
INCUBATION
(5-6 d)
E.O.P.
FIX, STAIN RECORD COLONIES
MUTANTS/10^5 CELLS
E.O.P.
DAY 8-16
REPORT INDUCED MUTANTS PER SURVIVING FRACTION OF CELLS


100
50
10
5
1
PERCENT SURVIVAL
0
2
4
6
8
SUN EXPOSURE (min)

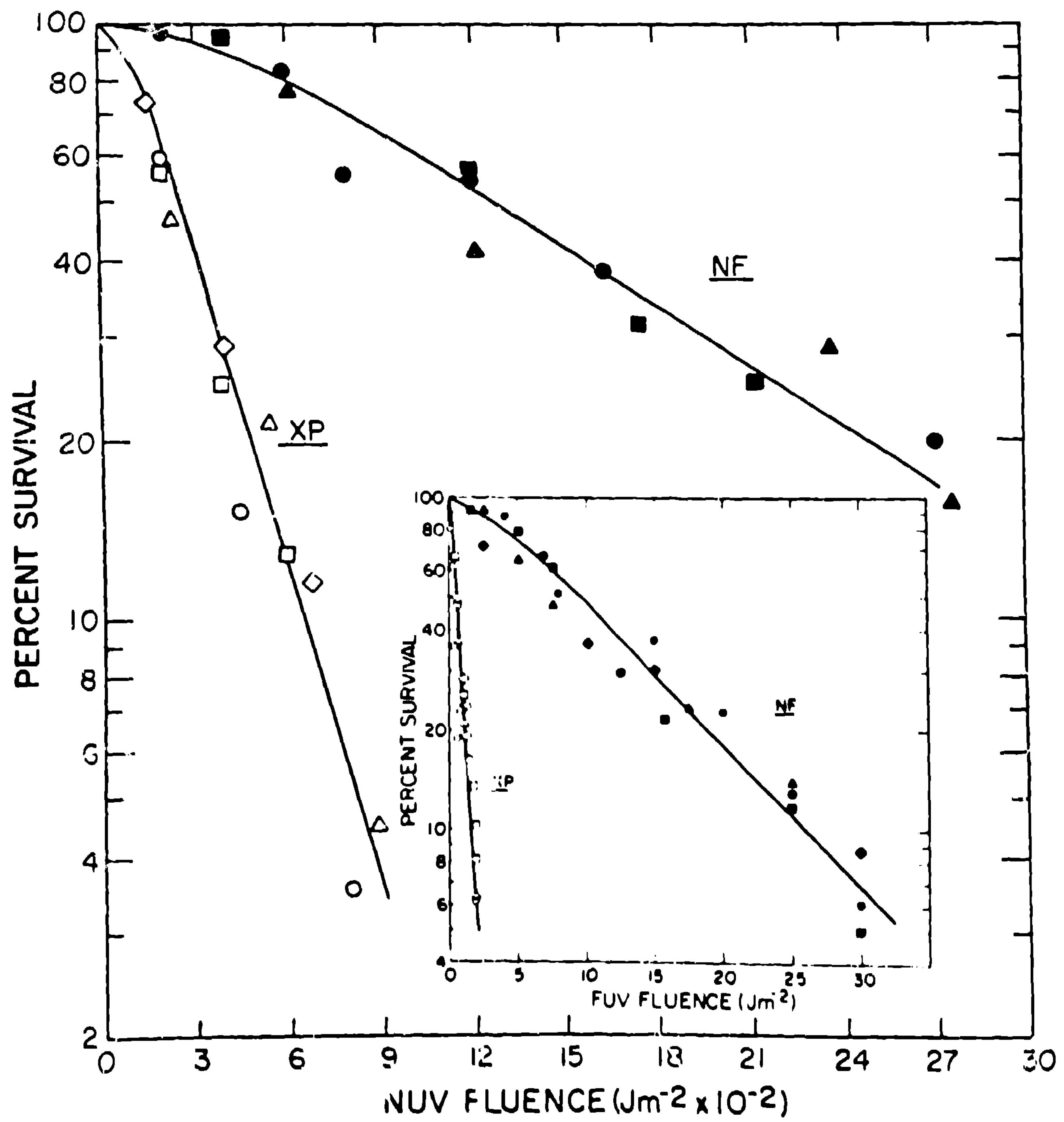

6 - TG^R MUTANTS PER 10^5 CELLS
50
40
30
20
10
100
50
20
10
5
PERCENT SURVIVAL


NUV FLUENCE ($Jm^{-2} \times 10^{-2}$)
0
6
12
18
24
100
80
60
40
20
10
8
6
4
PERCENT SURVIVAL
NUV
FUV
0
5
10
15
20
25
30
FUV FLUENCE (Jm^{-2})


UV FLUENCE (Jm^{-2})
0
10
20
30
40
50
30
20
10
0
6-TG^{R} MUTANTS PER 10^{5} CELLS
20
15
10
5
0
0
6
12
18
24
30
NUV FLUENCE ($Jm^{-2} \times 10^{-2}$)



6-TG^R MUTANTS PER 10^5 CELLS
20
15
10
5
0
100
50
10
5
PERCENT SURVIVAL



PERCENT SURVIVAL
NUV FLUENCE (Jm-2 x 10-2)
NF
XP
PERCENT SURVIVAL
FUV FLUENCE (Jm-2)
NF
XP