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Cytogenetic Effects of Shale-Derived Oils and Related By-Products in Mice

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CYTOGENETIC EFFECTS OF SHALE-DERIVED OILS AND RELATED BY-PRODUCTS IN MICE

by

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ABSTRACT

The cytogenetic effects of exposure to crude shale oil by either skin painting or intraperitoneal injection and transplacental exposure of embryos to water derived from surface retort processing of shale oil were analyzed. Exposure to crude shale oils from two sources, Paraho and Occidental, by skin painting had essentially no effect on the frequency of chromosomal aberrations in bone marrow cells. Intraperitoneal injection of the Paraho crude shale oil increased the frequency of chromosome damage in bone marrow cells at all three doses tested (0.5 ml, 1.0 ml, and 2.0 ml/kg). Metaphase analysis of cells from embryos at day 12 gestation from females that had been exposed to 1% Paraho retort water *ad libitum* from day 1 of gestation indicated that clastogenic compounds present in this water can cross the placenta and induce chromosomal damage in embryonic cells.

I. INTRODUCTION

In vivo cytogenetic analysis is an effective indicator of the potential of environmental agents as human health hazards. Although not all hazardous agents are detected as clastogens, agents that are clastogenic are usually health hazards (mutagens, carcinogens, or teratogens). It is the goal of this project to assess the extent of chromosomal damage induced by toxic agents associated with energy production, conversion, or utilization. Shale-derived oils contain many known mutagenic and carcinogenic compounds. Because of the expected development of an oil shale industry it is important to assess the occupational risk to workers and the hazards of toxic by-products of this technology that may be introduced into the environment. At present, two methods of approach are being used to assess the clastogenic effects of shale derived oils and their by-products. The first approach involves the established method of cytogenetic analysis of bone marrow metaphases from exposed mice. The second approach explores the potential of evaluating agents using transplacental exposure of pregnant mice followed by metaphase analysis of embryonic cells.

II. MATERIALS AND METHODS

The Paraho crude shale oil (PCSO) was from the Paraho Demonstration Project at Anvil Points, Colorado, and was obtained through the ORNL/EPA repository at Oak Ridge National Laboratory. The Occidental crude shale oil (OCSO) was from retort #6 of the Occidental Oil Shale Company at

Logan Wash, Colorado, and was obtained from the Battelle Pacific Northwest stock of materials. Both of these samples were non-upgraded crude shale oils.

The Paraho retort water (PRW) was also from the Paraho Demonstration Project at Anvil Points and was collected on site by a LASL field study at this site. It is a sample of water which had separated from the oil after it was placed in the large storage tanks. This water was untreated, unfiltered, and had not been kept cold during tank storage. Therefore, the sample represents a worst-case situation and is not representative of the retort water expected from more advanced technologies. The sample was filtered through Whatman #42 filter paper and a 0.10 μm Millipore filter to remove particulates before it was added to the drinking water.

Skin Painting: PCSO and OCSO were diluted using a 30% cyclohexane - 70% acetone vehicle. Fresh solutions were prepared each week and were stored at room temperature in dark glass vials. The dose used represented a weight-volume percentage of 80%. Each mouse was painted with 50 μl of crude oil mixture at about 9:00 a.m. every other day for either 12 days or 35 days. Each mouse received 40 mg of crude oil during each treatment. Male C3Hf/He mice, supplied by Dr. J. M. Holland of Oak Ridge National Laboratory, were used for the study. At the initiation of the experiment each mouse was shaved along the spine from hips to neck. The solutions were applied to the intrascapular region using a 50 μl MLA precision pipette. The mice were four weeks old when the study was initiated, and were divided into the following groups of three mice each:

Group A-PCSO, 12 days; Group B-OCSO, 12 days; Group C-PCSO, 35 days; Group D-OCSO, 35 days. Concurrent control groups were shaved but not treated. Two hours after the last exposure each mouse was given an intraperitoneal injection of 1 mg/kg colchicine to arrest cells at metaphase. Two hours after the colchicine injection the mice were killed by cervical dislocation and the bone marrow of one femur was processed for metaphase analysis.

Intraperitoneal Injection: Female Swiss (CD-1) mice, 8 weeks old, were housed in groups of 5 mice per cage. Immediately prior to each treatment PCSO was warmed to 37°C and diluted with olive oil to give final concentrations such that each mouse received 0.01 ml/gram body weight of the mixture. Control groups received the same volume of the olive oil solvent. The doses used were 0.5 ml, 1.0 ml, and 2.0 ml/kg PCSO. Exposure consisted of intraperitoneal injections given at 24-hour intervals for 3 consecutive days. The mice were killed by cervical dislocation 4 hours after the last treatment. To collect metaphases, colchicine (2 mg/kg) was injected 2 hours before killing. Chromosome preparations were made using the bone marrow from one femur.

Bone Marrow Metaphase Analysis: The marrow from one femur was flushed into a centrifuge tube containing 5 ml of Eagle's Minimal Essential Medium. After all the marrows from the treatment group were collected, the marrow suspensions were centrifuged at 1000 rpm for 5 minutes. The cells were processed by standard cytogenetic techniques using a 25-minute exposure to 0.075 M KCl hypotonic solution, followed by fixation in 3:1 methanol-acetic acid. Slides were prepared by flame drying and stained with Giemsa. A total of 100 metaphases were scored on each animal.

Transplacental Exposure: Randomly bred C3Hf/He females, 8-12 weeks old, were used throughout the study. Females were considered to be at day 1 of gestation on

the day a vaginal sperm plug was detected. At 9:00 a.m. on day 1 the pregnant females of the treatment group were placed in cages where their only drinking source was tap water containing 1% (v/v) PRW. The control group was maintained on tap water. On day 12 of gestation the females were killed by cervical dislocation. The uterus was removed, washed in saline, and placed in a Petri dish containing saline, and the embryos were freed from the placenta and surrounding embryonic membranes. Each embryo was then transferred to a separate centrifuge tube containing 2 ml of prewarmed (37°C) Eagle's Minimal Essential Medium with 0.5 µg/ml Colcemid (CIBA). Each embryo was dispersed into a cell suspension by gentle syringing, and the suspension was incubated at 37°C for about one hour. After this incubation period the suspension was centrifuged at 1000 rpm for 4 minutes, the supernatant decanted, and the cells resuspended in 3 ml of 0.075 M KCl (hypotonic solution). After 25 minutes the suspension was centrifuged and the cell button resuspended in 3 ml of 3:1 methanol-acetic acid fixative. Metaphase preparations made by the air-dry method were stained in 2% aqueous Giemsa. A total of 50 cells were scored from each of 6 embryos from each female.

III. RESULTS

Skin Painting

The frequencies of chromosomal abnormalities in the bone marrow of the shaved but untreated control mice were 0.01 chromatid breaks per cell and 0.04 centric fusions per cell. The aberration frequencies in the mice treated by skin painting of PCSO or OCSO for either 12 days or 35 days were within control levels. The range of chromatid breaks was from 0-0.01 per cell, and the range of centric fusions was from 0.03-0.05 per cell. The number of gaps observed was essentially the same for both treated and control groups. No other types of aberrations were observed.

Intraperitoneal Injection

Table I shows the aberration frequencies observed in the bone marrow of mice treated by intraperitoneal injection of three doses of PCSO and the solvent (olive oil) control. Each dose of PCSO increased the frequencies of both chromatid breaks and gaps. The effects of the 0.5 ml and 1.0 ml/kg doses were similar, showing about double the control level. The 2.0 ml/kg dose induced nearly five times more chromatid breaks than observed in control animals . Only one cell had more than one break, although some cells with one break also contained gaps or centric fusions.

Centric fusions are relatively common in somatic cells of mice. Because they can easily be confused with centromeric association, caution must be used when scoring these changes. Using strict scoring criteria, based on chromatid continuity in a single plane through the centromere, the frequency of centric fusions in the control mice was 0.02 per cell. The frequency of centric fusions after exposure to either 0.5 ml or 1.0 ml/kg PCSO was 0.03 per cell, and after 2.0 ml/kg it was 0.04 per cell. Because the frequency of centric fusions varies among mouse strains and individuals within each strain it is difficult to assess the significance of increases apparently induced by exposure to environmental agents.

The mitotic indices (Table I) indicate that the doses of PCSO used in this study do not severely inhibit cell proliferation. None of these doses demonstrated any overt toxicity in the treated mice. Doses of 3 ml and 4 ml/kg were quite toxic, however, and caused lethargy and abdominal bloating beginning the second day of treatment. One of the

TABLE I

CYTOGENETIC ABERRATIONS IN BONE MARROW OF FEMALE SWISS (CD-1)MICE AFTER INTRAPERITONEAL INJECTION OF PARAHO CRUDE SHALE OIL (PCSO)[a]

Dose PCSO	Total Cells Scored[b]	Number of Aberrations[c] b	B	g	G	Breaks per Cell	Gaps per Cell	Mitotic Index
control	500	4	0	11	2	0.008 ±0.008	0.026 ±0.009	4.54 ±0.59
0.5ml/kg	500	9	2	21	1	0.022 ±0.011	0.044 ±0.011	4.46 ±0.82
1.0ml/kg	500	10	3	20	5	0.026 ±0.011	0.050 ±0.007	3.62 ±0.88
2.0ml/kg	500	19	3	24	6	0.044 ±0.017	0.060 ±0.016	3.86 ±0.34

[a] Three injections of crude shale oil diluted in olive oil given at 24-hour intervals. Mice sacrificed 4 hours after the last injection.

[b] A total of 100 cells from each of 5 mice.

[c] b-chromatid breaks; B-isochromatid breaks; g-chromatid gaps; G-isochromatid gaps.

five animals from each of these groups died prior to the third injection. Therefore, these higher doses were not used for this study.

Transplacental Exposure

Table II shows the cytogenetic effects observed in metaphase cells of embryos from females exposed to 1% PRW *ad libitum* from day 1 of gestation until sampling on day 12. The frequencies of chromatid breaks, isochromatid breaks, and exchanges (other than centric fusions) were increased in the exposed embryos. There was essentially no difference in the frequency of centric fusions between control and treated embryos (0.027 vs. 0.033 per cell).

IV. DISCUSSION

Both the Paraho and Occidental crude shale oils are carcinogenic to C3Hf/He mice when applied by the same skin painting method used for this investigation.[1] The dermal and histological effects of these oils on mouse skin have also been characterized.[2] It is not surprising, however, that exposure to crude shale oils using skin painting did not increase the frequency of chromosomal aberrations in bone marrow. Most of the histological and carcinogenic effects observed after skin painting appear to be localized. There does not appear to be any substantial systemic toxicity after short-term exposures to crude shale oils by skin painting. Therefore, the clastogenic components would not be expected to reach the hematopoietic tissues in doses high enough to induce chromosome damage. Because of the suspected relationship between malignancy and chromosomal aberrations, the feasibility of applying cytogenetic analysis to epidermal cells from painted areas is being explored for further studies.

Intraperitoneal injection is a direct method to test for the effects of systemic exposure. To date, only PCSO has been tested using this route of exposure. The Swiss (CD-1) strain of mice was used for this analysis because we are more familiar

TABLE II

CYTOGENETIC ABERRATIONS IN DAY 12 EMBRYOS FROM FEMALES EXPOSED TO 1% PARAHO RETORT WATER (PRW) AD LIBITUM

Exposure	Female	Total Cells Scored[a]	Number of Aberrations[b] b	B	xc	gaps
Control (tap water)	I	300	9	0	0	14
	II	300	6	0	0	15
	III	300	8	0	0	18
	IV	300	6	0	0	14
	V	300	7	0	0	17
		mean/cell	0.024 ±0.004	0	0	0.052 ±0.006
1% PRW (in tap water) ad libitum	I	300	13	3	4	26
	II	300	23	9	0	27
	III	300	17	3	3	26
	IV	300	22	1	1	31
	V	300	19	1	3	27
		mean/cell	0.063 ±0.013	0.011 ±0.011	0.007 ±0.005	0.091 ±0.007

[a] A total of 50 cells scored on each of 6 embryos from each female.

[b] b-chromatid breads; B-isochromatid breaks; xc-exchanges other than centric fusions.

with the cytogenetic response of this outbred strain following intraperitoneal injection of environmental agents (J. Meyne, unpublished data). The doses of PCSO used in this study were clearly clastogenic. Although intraperitoneal injection is not directly relevant to routes of human exposure, this investigation does indicate that acute systemic exposure to PCSO can damage chromosomes. The data from this study combined with the results of the tumor induction studies[1] in mice demonstrate the need for additional investigations to determine the potential of crude shale oils as a health hazard and to establish exposure limits and safety procedures for the developing shale oil technology. Future studies will include cytogenetic analysis after intraperitoneal injection of OCSO and hydrotreated PCSO.

Cytogenetic effects of transplacental exposure to environmental agents is a relatively unexplored area. Only a few chemicals and irradiation have been analyzed using this method. With the increasing number of women of reproductive age employed in chemical and energy-source industries, and the concomitant risks from exposure to environmental pollutants, the potential effects of transplacental exposure on the embryo must be considered when evaluating health effects of new products or new technologies. Surface retorting of oil shale produces large quantities of retort water. If the disposal methods are inadequate, retort water residues could contaminate drinking water sources in specific geographical areas. Results of our study using

exposure of pregnant females to PRW indicate that clastogenic compounds within this water can cross the placenta and induce chromosomal aberrations in the embryos. Further studies are planned to assess the effects of PRW ad libitum in the bone marrow of non-pregnant females.

Teratology studies on fetuses from Swiss ICR/DUB females given from 0.1% to 1.0% PRW ad libitum during gestation have shown a significant increase in the frequency of congenital malformations.[3] The preliminary cytogenetic study summarized in this report used C3Hf/He mice, but the same protocol is currently being conducted with Swiss ICR/DUB mice. The data from this investigation can then be compared to those of the teratology study. By coordinating these two methods of analysis we can provide cytogenetic information concerning the effects of transplacental exposure to PRW on the embryo and also examine possible relationships between clastogenic events and the pathogenesis of congenital malformation.

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