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MASTER

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**Studies of the Biology and Toxicology
of Oil-Shale Materials
L. M. Holland**

Introduction:

Most fossil fuels are made up of biologically active compounds which can, under the proper circumstances, lead to one or more distinct toxic effects. The data available to establish the potential health effects of a U. S. oil shale industry are meagre and cannot yet be used for comparative risk analysis. Historical evidence, including both clinical observations and experimental results, indicate a carcinogenic potential associated with the crude oils and oil by-products but a relatively low respiratory hazard. This historical experience is drawn largely from foreign industries utilizing geologically different shales and technically different extraction processes.

Current research dealing with the biological effects of oil shale materials offers both an opportunity and a dilemma. We have, for one of the few times in history, the chance to assess biological and health effects before an industry is developed and, by extension, to help identify the hazards and contribute to their mitigation. The dilemma arises from the complexity of the potential exposure and the materials involved. Classic animal studies with complex mixtures can identify certain effects and are germane because they can deal with the complete material. Contributions to the science of control technology, however, will be better served by a combination of chemical definition, in vitro assays and in vivo studies applied to significant compound classes or fractions.

The simple recognition of a toxic effect by a particular process-stream product or one of its sub-mixtures is not useful unless the probability for, and mode of, exposure are considered at the same time. Conversely, it is not enough to consider only the effects on the presumed target organ or tissue without regard to metabolic pathways, potential late effects and developmental and genetic phenomena.

This paper will discuss biological assessment of materials in relationship to probable exposure routes and attempt to describe the importance of the more fundamental bioassays in predicting eventual hazard.

Cutaneous Exposure

Historically, skin cancer and various benign dermatoses have been the occupational diseases most often associated with shale oil production and use. In 1922 Alexander Scott described the various manifestations of skin disease found in Scottish oil shale workers and discussed the etiology and anatomical location of lesions and suggested prophylactic measures (1). He concluded that direct contact with shale oil or its byproducts was the cause of occupational skin disease and further noted the influence of work assignment on the type and location of clinical lesions. Several investigators in the United Kingdom developed experimental evidence of the carcinogenic potency of various shale oil products (2,3,4) and Bogovski and others in Estonia have studied shale oil carcinogenesis using several in vivo systems (5).

Many studies, particularly in the Soviet Union, have relied upon the presence of benzo-a-pyrene (BaP) as a sort of indicator of carcinogenic potency. This approach has been shown to be unreliable in dealing with the complex mixtures produced from oil shale (6).

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All crude retort oils tested in an American Petroleum Institute (API) mouse skin painting program were positive for carcinogenesis with an average latency of 20-28 weeks to tumor appearance compared to a 38 week latency for BaP controls (7).

In a comparative study of two coal-derived liquids, a shale oil from a simulation of MIS retorting and a blend of natural petroleums, all oils, except the natural petroleum blend, demonstrated carcinogenicity and dermatotoxic potency (8). Mice were exposed 3 times/week for 22 weeks and then observed for an additional 150 days. This somewhat foreshortened exposure regime exhibited little difference in tumor latency between the synthetic oils and placed the shale oil between the two coal-derived liquids on a basis of comparative potency. In a second study involving a crude oil, an upgraded crude and several middle distillates (jet fuel, diesel fuel, etc.) only the crude oils and a hydrotreating residue were found to be carcinogenic. However some of the middle distillates proved to be ulcerogenic at high doses. Differences in carcinogenic potency have been observed between shale oils from different processes in an experiment where natural petroleums were also found to vary in carcinogenic effect (9). In a second, still incomplete, study comparisons are being made between two crude shale oils and the hydrotreated product of one. Early results indicate a nearly parallel carcinogenic and dermatotoxic potency among the non-upgraded retort oils but a diminished effect from the hydrotreated product oil. Wilson has reported on the acute epidermal changes observed after daily application of high-percentage dilutions of shale oils. In this and later studies (10) ulcerogenic lesions were observed which in some instances became large nonhealing areas completely devoid of epithelium.

While inflammatory cytotoxic effects observed in mouse skin painting studies are important in themselves and provide some information about the irritative potential of the various oils, they also considerably cloud the carcinogenicity issue. If test materials are applied at strengths or frequencies that lead to significant epithelial cytotoxicity very little sense can be made of the data even if tumors are observed. It should be borne in mind that the skin is not just a protective sheet of cells, but is a complex organ with distinct metabolic functions and rich enzymatic activity. It is probable that components applied to the skin are enzymatically activated and may be selectively absorbed through the skin in a way that may lead to systemic toxicity or to tumorigenesis in distant organs. Recent experiments have demonstrated an apparent nephrotoxicity in mice receiving skin applications of both oils and distillation products (11).

Rodent skin painting schemes are empirical tests and are particularly applicable to such complex mixtures as shale oil, but they should not be used to condemn specific products or processes out of hand. More studies that attempt to determine which components of the mixture are active and in what way (i.e. initiation, promotion, antagonism) are needed and should be conducted in a manner that takes into account such factors as host response, effect on tumor angiogenesis, inhibition of host activating systems, mouse strain differences and the like.

Clinical and experimental evidence ascribes a carcinogenic potency to shale oil that is higher than that seen with most natural petroleum and lower than that observed with some coal-derived liquids. Most of the epidemiological evidence for a high skin cancer incidence in the industrial work force is drawn from a time when knowledge of industrial hygiene was

less sophisticated and personal hygiene was of less social importance. These observations are meaningful only when they are considered along with the data from studies of modern oil refinery workers where the incidence of skin cancer has been shown to compare favorably with that observed in other worker groups.

Pulmonary Exposure:

Mining and crushing of raw shale and the disposal of spent shale all have the potential for releasing fine dust particles in a size range known to result in pulmonary retention. The size of shale ore used in surface retorting varies with the process and the resulting spent material will, because of differences in particle size, present varying pulmonary hazards. Much of the concern over pulmonary hazard is related to industrial environments but the possible release of certain criteria pollutants (No_x , So_x) and an increase in dusty conditions in the area could lead to regional problems. Of particular interest is the vertical modified in situ (VMIS) technique where two dozen or more large retorts will be burning simultaneously in mine drifts adjacent to underground areas in which new retorts are being prepared. This special set of conditions presents the possibility of unique combined exposures (e.g. dusts, fugitive organic vapors, machinery exhausts and the like).

Chronic bronchitis, mild pneumoconiosis and emphysema have been identified in autopsy material from Estonian oil shale workers. The relatively low incidence is attributed to the low free silica content of kukersite (Estonian shale) and to the low concentration of dust in the mines (12). An occupational disease of the upper respiratory tract (nasopharynx and major sinuses) which is apparently somewhat dependent on job assignment has been described (13). In this study 7.7% of the oil

shale miners suffered from chronic hypertrophic rhinitis while 7.6% of workers coming in contact with oil shale ash (soot) suffered from atrophic rhinitis. The latter, more severe disease is attributed to the nature of the ash and the additional exposure to fugitive gases from the recovery processes. The clinical pattern apparently resembles that seen after inhalation of cement or superphosphate.

Most of the current experimental animal studies in the United States have concentrated on the effects of the dusts administered either as inhaled aerosols or by repeated intratracheal instillation. Coomes has reported on two experiments involving pulmonary responses to both raw and spent shale materials. One of these was a so called "total exposure" experiment where hairless mice were housed on shale used as bedding; no lung tumors have been reported. In the second experiment, Syrian hamsters were subjected to repeated intratracheal instillations of either raw or spent shale. Apparently no tumors were observed in this experiment (14). Recent inhalation studies using Syrian hamsters exposed to high concentrations of either raw or spent shale over a 16 month period have been reported. None of the animals exhibited tumors but it was noted that spent shale appeared to cause a more pronounced thickening of the interalveolar tissues, occasional fibrotic scars and extension of bronchiolar epithelium (15).

The API has sponsored a study in which pregnant female rats were exposed to raw shale dust. No embryotoxic or teratogenic effects were observed (16).

Few inhalation experiments attempt to measure all of the possible effects in a single all encompassing design. When dealing with highly complex mixtures it may be best to first answer empirical questions before

initiating more refined studies. This approach first identifies an effect before defining the mechanisms leading to that effect. The choice of a species can influence the outcome of an experiment. The Syrian hamster has become a popular model for either chemically or radiation induced tumorigenesis but is not regarded as the species of choice for pulmonary fibrogenesis experiments. Studies using intratracheal administration have their place but cannot substitute for inhalation exposures which are more physiological. Measurement of deposited (or retained) dose is difficult in inhalation studies (17) and is affected by such things as total concentration, mode and frequency of exposure and nature of the aerosol.

Systemic Toxicity and Genetic Effects in Animals

The question of general toxicity and potential genetic effect is more difficult to define in intact animal systems; not because appropriate tests do not exist but because routes of exposure, dose levels and opportunity for contact are harder to predict. For example, while it may be interesting and scientifically valuable to determine an LD₅₀ for a product oil, it does not necessarily help in the assessment of the industry since the opportunities for that level of exposure by a relevant route are practically nonexistent.

The most immediate question in this area remains the potential for water contamination. Both surface waters and regional aquifers are vulnerable to contamination from product oils, process effluents, spent or partially processed shales and other industrially related materials. From the industry standpoint it is important to know not only if the contaminated waters are biologically active but what the contaminants are, their origins (both generic and process induced) and what procedures are necessary for clean-up.

Among the systemic effects that might result from contamination of regional water supplies is disturbance of the germinal tissues either as a mutation, a failure of reproduction or a direct effect on fetal development. Gregg has measured the embryotoxicity of one untreated retort or product water and identified an unusual number of defects of the palate in mouse embryos. While no significant changes in fetal numbers and weights were noted, the lesions of the palate exhibited a dose response. No overt signs of maternal toxicity were noted (18). Measurements of the direct toxicity and teratogenic potential of the so-called Omega-9 water associated with true in-situ retorting near Rook Springs, Wyoming have been largely negative. This material is apparently a mixture of ground water and water associated with the in-situ burn (19).

Other tests of genetic damage and reproductive fitness which should be utilized include dominant lethal assay, oocyte depletion, sperm head abnormalities and chromosome aberrations. Some work has begun in this area but the experiments have been limited in scope.

Careful attention should be given to the possibility of contamination of soil and water with toxic trace elements and to the potential for bioaccumulative phenomena. Some of these materials do not reveal their biological activity easily with the result that chemical detection must be more heavily relied upon for the identification of hazard.

In Vitro Testing

A number of useful assays have been developed for measuring a general effect or as predictors of more specific alterations in intact mammals (including man). Chief among these is the Salmonella histidine reversion system (Ames assay) which tests for mutagenesis and putative carcinogenesis. Several investigators have applied the Ames assay to

either whole shale oils or selected fractions (20). Some of the results with whole oils have been equivocal but several studies indicate that the highest mutagenic activity resides in the basic fraction. Unfortunately the vehicles used in the bacterial assay are usually themselves organic solvents with the result that defacto fractionations are inherent in the procedure. When a more active fraction is identified the information may have some impact on eventual control procedures if it can be demonstrated that changes in process conditions or that additional product treatments reduce the activity. However, because of possible synergisms, antagonisms and masking effects the sum of the activity of fractions is often different than that of the original mixture. Waters, however, do not require solvent vehicles and the results derived from the Ames systems are more straightforward. Barnhart has reported the relative mutagenicity of several untreated retort waters and found that the potency is related to that observed in the associated oil (21). The next logical step is to determine what compound classes in the water are most biologically active.

Other assays involving both cytotoxicity and mutagenesis and employing mammalian cells are being used. Ideally a suite of relevant samples should be tested using as many comparable systems as possible in an effort to overcome the lack of universal coverage by any one assay. The probable enrichment of some shale materials with heavy metals is an example of this need. The arsenicals are known human carcinogens which are refractory to testing by either the bacterial systems or by in vivo methods (22).

The science of cellular genetics is one which can be employed both in vivo and in vitro. The frequency of certain chromosomal alterations (sister chromatid exchange, endoreduplication) have been studied in relation to some oil shale materials but the implications of increased

rates are unclear. Cytogenetic changes are generally assumed to be indicative of longer term effects but a quantitative relationship with neoplasia, generational defects or general toxicity is ill defined.

Bone marrow cells have been analysed for chromosomal aberrations following skin exposure to either surface process or modified in situ crude oil. No effects were observed. Intra-peritoneal injection of one shale oil increased the frequency of chromosome damage at all three doses tested. Metaphase analysis of cells from mouse embryos at day 12 of gestation from females which had been exposed to untreated retort water from day 1 of gestation indicated a transplacental movement of clastogenic compounds. Chromosomal damage in embryonic cells resulted from exposure of the dam to 1% retort water (23).

Discussion

A number of generalizations can be made based on historical evidence and trends in current research. It should be borne in mind that both the Scottish and the Estonian shales and the technologies employed to extract them differ in significant ways from the raw materials and proposed techniques to be employed in the U.S. Our level of knowledge can be summarized as follows:

1. Most fossil liquids possess carcinogenic potential. The potency of shale oils, as a general class, is probably somewhat higher than most natural petroleum and perhaps lower than many coal-derived liquids.
2. The carcinogenic potency ascribed to crude shale oil is significantly lowered by hydrotreating.

3. Raw and spent shale dusts have not been shown to be carcinogenic in the lung. The questions related to fibrogenic or obstructive lung disease have not been adequately studied.
4. Certain proposed U.S. technologies may present an inhalation hazard that is yet to be defined.
5. The contamination of either ground or surface waters with biologically active materials is probable and strong control-technology efforts will be required. Existing water clean-up techniques may be sufficient but this can only be determined by continued biological testing as the industry scales up. In this area the shorter term in vitro assays will be of particular benefit.
6. Trace element (particularly heavy materials) contamination and bioaccumulation phenomena should be studied as a continuing effort as the industry intensifies.
7. The biological activity of certain oil shale products may be process-chemistry dependent. The historical evidence of the influence of process temperature is particularly strong. This is the area where cooperation between process engineers, control technologists and biologists can be most fruitful.
8. Studies related to the toxicity of shale oil end-products have just begun and should be accelerated. (e.g. petrochemical feedstocks, fuel combustion products, etc.)

Toxicological experiments and bioassays can provide information for use by control technologists, industrial hygienists and others who are concerned with minimizing health and environmental impacts but the data should not be misused or lifted out of context by those who have a poor

understanding of the entire process. The observation that fossil fuels, including natural petroleums are carcinogenic is nothing new. It is to the credit of the industry that modern refining techniques have almost totally mitigated that effect. There is little question that when oil shale resources are developed both extraction and use can be accomplished in a sensible, safe manner and that the hazards can be controlled as they are identified. The identification and mitigation of health hazards will depend upon continued close cooperation between industry, state governments and the federal government.

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