Bull. Environm. Contam. Toxicol. 23,747-752 (1979)

Concentration-Dependence of the Effect of an *In Situ* Oil Shale Retort-Produced Water on Metabolism

K. F. Nelson, D. S. North, and D. S. Farrier[1]

School of Pharmacy, University of Wyoming, Laramie, Wyo. 82071 and
[1]Laramie Energy Technology Center, U. S. Department of Energy, Laramie, Wyo. 82071

Significant quantities of waste waters are produced during thermal retorting of oil shale to produce shale oil. The initial water co-produced with the shale oil is referred to as retort water (FARRIER et al. 1978a). Retort water is derived primarily from water released by dehydration of oil shale minerals and water formed as a byproduct of combustion of organic matter during retorting. In the minimum case, the amount of retort water derived from these two sources will approximately equal the amount of oil produced. During in situ (below ground) oil shale processing the quantity of retort water may be considerably enhanced because of the recovery of ground water intruding into the retorting zone (FARRIER et al. 1978a). An illustration of this is the water-to-oil ratio of 22.2 obtained during the Laramie Energy Technology Center's 1976 Rock Springs Site 9 true in situ oil shale retorting experiment (FARRIER et al. 1978a, LONG et al. 1977).

During the conduction of the Rock Springs Site 9 experiment a large volume of retort water was collected to support environmental research studies (FARRIER et al. 1977). This sample was designated "Omega-9 retort water." Detailed compositional characterisitcs of the Omega-9 water have previously been reported (FOX et al. 1978, STUBER and LEENHEER 1977, FELIX et al. 1977). Briefly, the Omega-9 water has the following characteristics: pH 8.6, 1003 mg/l total organic carbon, and 30,300 mg/l of total dissolved solids which are principally bicarbonate, sodium, ammonium, thiosulfate, sulfate, chloride, and carbonate.

Although the Omega-9 water cannot be ascribed as typical of in situ oil shale retort waters in general, its availability has supported numerous investigations into the treatment, disposal, use, and effects of such water (FARRIER et al. 1978a, FARRIER et al. 1978b). An animal toxicity evaluation and interpretation of the occupational safety aspects of Omega-9 water has been reported (HEPLER et al. 1979). In a previous paper (NELSON et al. 1978) we reported that consumption of undiluted Omega-9 water by 60-80 g Holtzman rats led to an increased rate of metabolism of hexobarbital in vivo and in vitro, an increased rate of metabolism of ethylmorphine in vitro, and an increased level of cytochrome P-450 activity. These observations indicated that

0007-4861/79/0023-0747 $01.20

Omega-9 water induced liver enzyme activity. The purpose of this study was to determine if the induction effect is concentration related.

EXPERIMENTAL

Male Holtzman (Holtzman Company, Madison, Wisconsin) strain rats weighing 60-120 gm. were used for all experiments. Control rats were fed normal solid food diet and tap water ad lib. Experimental animals were fed normal solid food diet (Purina Rat Chow) and Omega-9 water diluted to various concentrations with tap water ad lib.

Each group of rats was kept on their respective nutritional source and liquid for four days. Experiments were carried out on the fifth day. All data were analyzed for statistical significance using the procedure of SCHEFFE (1959).

Hexobarbital - In Vivo

Hexobarbital sodium was injected intraperitoneally (ip) at a dose of 125 mg/kg into control rats and rats drinking Omega-9 water on the fifth day of experimentation. Sleep time was calculated for each rat in each group from the time of loss of righting reflex until that reflex was regained.

In Vitro

On the fifth day control and experimental animals were sacrificed by decapitation and their livers immediately excised. The livers were blotted dry, weighed, and placed in beakers containing cold 0.25M sucrose. The beakers were then placed in an ice bath.

Liver homogenates were prepared using not less than three pooled livers from each group. The livers were minced and five volumes of cold 0.25M sucrose solution added forming a 20% w/v mixture. Homogenization was carried out using a motor driven teflon pestle and glass homogenizing tube and grinding through ten passes. The soluble fraction of the homogenates was obtained by centrifugation (COCHIN and AXELROD 1959). Protein values were obtained using the method of LOWRY *et al.* (1951).

RESULTS

Hexobarbital - In vivo

The comparative duration of hypnosis by hexobarbital sodium after (ip) injection of 125 mg/kg in control rats and rats drinking diluted Omega-9 water may be seen in Table 1.

TABLE 1

Concentration Dependence of Induced Hexobarbital Metabolism from Omega-9 Water (Oral Ingestion)

Group	No. of Rats	Duration of Hypnosis Mean ± S.E. (minutes)	Statistical Significance
Controls	10	62.6 ± 1.18	
25% Omega-9 Water to Distilled Water	10	50.6 ± 0.803	$p < .001$
50% Omega-9 Water to Distilled Water	10	45.8 ± 0.887	$p < .001$
75% Omega-9 Water to Distilled Water	10	34.6 ± 1.31	$p < .001$
100% Omega-9 Water	10	43.9 ± 2.08	$p < .001$

These data indicate that the amount of induction is proportional to the concentration of Omega-9 water except in the case of 100% Omega-9 water.

In Vitro

The enzymatic degradation rates of sodium hexobarbital in vitro by liver homogenates from control and experimental animals exposed to various concentrations of retort water is given in Table 2. These data indicate that with the exception of the 25% concentration of Omega-9 water, each concentration of Omega-9 water significantly increased the metabolism of hexobarbital in liver microsomes obtained from rats exposed to Omega-9 water.

Ethylmorphine

The enzymatic degradation rates in vitro by liver homogenates from control and experimental animals exposed to various concentrations of Omega-9 water is given in Table 3. These values can be compared to the mean values for metabolism of ethylmorphine in controls, which was 0.134 M/cc with a 95% confidence interval between 0.125 M/cc and 0.143 M/cc. From this data it can be seen that, with the exception of the 50% Omega-9 concentration, each concentration significantly increased ethylmorphine demethylation activity in liver microsomes obtained from rats maintained on Omega-9 water.

TABLE 2

In Vitro Metabolism of Hexobarbital in Rats
With Various Concentrations of Omega-9 Water

Group	No. of Rats	Rate of Na Hexobarbital Metabolized Mean ± S.E. (μM/g. liver/hr.)	Statistical Significance
Controls	8	0.70 ± 0.015	
25% Omega-9 to Distilled Water	8	0.80 ± 0.038	$p < .001$
50% Omega-9 to Distilled Water	8	0.83 ± 0.020	$p < .001$
75% Omega-9 to Distilled Water	8	0.92 ± 0.030	$p < .001$
100% Omega-9 Water	8	0.92 ± 0.018	$p < .001$

TABLE 3

In Vitro Metabolism of Ethylmorphine in
Rats with Various Concentrations of Omega-9 Water

Group	No. of	Ethylmorphine Metabolism Mean ± S.E. μmoles/cc	Statistical Significance
Controls	8	0.134 ± 0.001	
50% Omega-9 to Tap Water	8	0.131 ± 0.002	$p > .05$
65% Omega-9 to Tap Water	8	0.157 ± 0.005	$p < .05$
75% Omega-9 to Tap Water	8	0.160 ± 0.005	$p < .05$
85% Omega-9 to Tap Water	8	0.235 ± 0.005	$p < .05$
100% Omega-9	9	0.199 ± 0.0138	$p < .05$

DISCUSSION

Increase in enzyme induction was almost directly proportional to the concentration of Omega-9 water. This was shown utilizing *in vitro* and *in vivo* studies on hexobarbital and an *in vitro* determination of ethylmorphine demethylation. Peak induction (Table 1), as measured by duration of hypnosis by hexobarbital, occurred at a concentration in the vicinity of 75% Omega-9 water in distilled water. Peak metabolism of hexobarbital in the microsomal fraction, and therefore enzyme induction, occurred at a concentration again below 100% (Table 2). Finally peak metabolism of ethylmorphine (Table 3) also occurred at a concentration below 100%. The fact that peak induction occurs somewhere below 100% Omega-9 water would seem to indicate that the undiluted effluent may be causing either cellular destruction or enzymatic degradation within the liver. This conclusion is also consistent with the toxicological studies done on the developing rat (CULVER *et al.* 1979); however, other explanations could be valid.

Further research will be necessary to determine what components of the Omega-9 water are responsible for the induction. This can only be assessed after tier-fractionation of the water into organic and inorganic fractions is completed.

This preliminary study shows that dilution lower enzyme induction. This study should facilitate interpretation of other toxicological data (HEPLER *et al.* 1979).

ACKNOWLEDGEMENTS

The authors would like to express appreciation to Winthrop Laboratories for their generous supply of hexobarbital sodium used in this study. This research was supported by the U.S. Department of Energy Laramie Energy Technology Center under Contract #EY-77-C-04-3913 with the Rocky Mountain Institute of Energy and Environment, University of Wyoming, Laramie, WY 82071.

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