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Systemic Toxicity of Coal Liquefaction Products: Results of a 14–Day Dermal Exposure

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Increasing energy demands, coupled with rising prices and an unstable world oil market have stimulated international interest in developing alternative sources of fuel. Direct coal liquefaction processes (CLP) hold great potential for Canada because of its large coal reserves. The conversion of coal to liquefied fuels results in many fractions of differing hydrocarbon content and includes many toxic substances such as polynuclear aromatic hydrocarbons. Since the major route of occupational exposure would be via the dermal route and since studies of systemic toxicity following dermal exposure are lacking, (USDOE, 1980; USDHEW, 1977) preliminary studies were conducted on the toxicity of SRC-II process coal liquefaction products applied dermally to the rat.

MATERIALS AND METHODS

Samples of the light (L), intermediate (I), and heavy, (H) fractions produced during the SRC-II coal liquefaction process, were kindly provided by the Sandwell Beak Research Group (Mississauga, Ontario, Canada). Diesel Fuel (D) was purchased from an Esso Gasoline Station. The various fractions had the following physico-chemical characteristics (L), BP < 200°C, Total aliphatics = 8.5%, total benzenoids = 12.2%, total phenolics = 1.3% and total polynuclear aromatics = 74.9%; (I), BP 200-345°C, total aliphatics = 5.8%, total benzenoids = 12.2%, total phenolics = .12% and total polynuclear armomatics = 89.8%; (H), BP 345-455°C, total aliphatics = 1.8%, total benzenoids = 0.2%, total phenolics = 1.8% and total polynuclear aromatics = 94.2%.

Male and female Sprague-Dawley rats weighing 200 ± 25 grams were purchased from Charles River Canada Inc., and were acclimatized to laboratory conditions (temperature 20-22°C, relative humidity 40-60%, light/dark cycle of 12 hours) for 1 week before treatment. They were housed individually in stainless steel cages and were given food (Rodent Laboratory Chow, Ralston Purina) and water ad libitum.

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The study was designed according to OECD guidelines for range finding studies preliminary to a 28 day sub-acute study. Five treatment groups containing 5 males and 5 females were dosed dermally 5 days per week for 2 weeks at a rate of 1000 mg/kg bw/day. The area of application was a shaved area on the inter-scapular region approximately 6 cm^2 and application was via a Hamilton microlitre syringe. The area of application was not occluded after treatment. Group 1 served as an internal control and was only shaved. Group 2 received the light fraction, group 3 the intermediate fraction group 4 the heavy fraction. Group 5 received diesel fuel and acted as a postive control; Group 6 served as an outside control and was housed in a separate animal room. The animals were observed for signs of overt toxicity during the 10 days and body weights recorded every two days.

On day 15, the animals were anesthetized with ether to the surgical level and exsanguinated via the abdominal aorta. All animals were examined grossly at the time of the necropsy and major organs weighed. Serum biochemical determinations were made using a Technicon microanalyser (Model 12/60) and included sodium, potassium, inorganic phosphate, total bilirubin, alkaline phosphatase, Aspartate amino transferase (AsAT), total protein, calcium, cholesterol, glucose, uric acid and lactic dehydrogenase.

Approximately 2 grams of liver was homogenized in 2.5 vol. of 1 molar Tris/KCl and microsomal enzyme activities were determined by an automated method based on the methods of Fouts (1963) for microsomal aniline hydroxylase (AH), Cochin and Axelrod (1959) for the aminopyrine demethylase (APDM), and Burke and Mayer (1974) for ethoxyresorufin deethylase (ER) activities. Liver protein concentrations were determined by the biuret method (Gornall et al., 1948).

The following tissues were fixed in buffered formalin and were examined microscopically: brain, pituitary, liver, adrenal, thyroid, parathyroid, thymus, lungs, trachea, bronchi, thoracic aorta, esophagus, gastric cardia, fundus and pylorus, duodenum, pancreas, colon, kidney, spleen, bone marrow, mysenteric and media stinal lymph nodes, testes, epididymis, skeletal muscle, skin and heart.

Hematological parameters examined in this study included hemoglobin concentration, packed cell volume, erythrocyte count, total and differential count of leukocytes, mean corpuscular volume and mean corpuscular hemoglobin concentration.

Serum proteins were separated on agarose gel using Paragon SPE Electrophoresis Kit (number 65590) with the Paragon Electrophoresis System.

Statistical analyses of the data were carried out using one way analysis of variance. When significant treatment differences occurred ($P < 0.05$), Duncan's multiple range test was applied to the data to determine the group or groups that were different from the controls (Nie et al., 1977).

RESULTS & DISCUSSION

During the first week of treatment those animals receiving the Light Fraction (L) showed normal behavior but by the end of the week the animals became less active and there was evidence of skin irritation. Dosing with the Intermediate (I) fraction led to skin irritation within two days, followed by keratinization. Their urine became very dark. The Heavy Fraction (H) caused the animals to become docile with no overt signs of toxicity. Diesel fuel (D) elicited a response very similar to the I fraction.

In the second week, animals treated with (L) showed severe irritation and some had bleeding at the area of application. Animals on (I) began to lose weight and had severe damage with peeling skin at the area of application. The skin of animals treated with (H) was unaffected. Animals treated with (D) resembled those treated with (I).

Males and females treated with (L), (I), and (D) had a significantly lower body weight than the control (Table 1). At necropsy males in groups (L) and (D) were found to have a reduction in liver weight while the females in (H) and (I) had significantly higher liver weights (Table 1), however both males and females in groups (I) and (H) only showed higher liver/bw ratios.

Table 1 shows the serum biochemical parameters which were altered by treatment. The females in groups (I) and (D) showed a significant reduction in serum glucose, while those females on (L) showed a similar although not significant tendency. This was not seen in male rats. Males in Group (D) showed a significantly lower cholesterol content and in females, the tendency although not significant was also to reduced cholesterol levels in (D). Serum protein content in males in (L) (I) and (D) were significantly lowered, but the females were unaffected. Induction of ethoxyresorufin deethylase (ER) occurred in Group (H) for both sexes.

Hematological changes are shown in Table 2. Generally for all dose groups a reduction was noted in hemoglobin content, hematocrit and red cell count. A significant polychromasia was seen in groups (L), (I) and (D) in both sexes. There was a significant reduction of lymphocytes in these same groups. Generally the effects were the same for both males and females indicating relatively little sex difference in hematological effect.

Histopathological examination revealed that both male and female rats exposed to CLP's had marked hypoplastic and inflammatory changes in the skin with very mild changes in parenchymal organs. Bone marrow changes consisted of increased cellularity with myeloid hyperplasia and increased production of neutrophils, apparently as a response to the skin injury at the application site. Rats dosed with (H) showed increased liver weights consistent with the induction of ER activity in the liver

Table 1 Gross Pathological and Biochemical Parameters in Rats Dosed with CLP's at 1000 mg/kg[a]

	Gain body weight (g)	Liver weight (g)	Glucose mg/100 ml	Cholesterol mg/100 ml	Total protein g/100 ml	Liver protein mg/g	ER(nmol/min. /mg protein)
Male Group							
Control	82 ± 14.5	12.5 ± 1.5	142 ± 14	89.2 ± 16.3	7.5 ± 0.35	21.2 ± 2.1	0.88 ± 0.28
Outside Control	84 ± 15.8	12.4 ± 0.6	152 ± 10	88.2 ± 7.5	7.6 ± 0.23	23.0 ± 3.5	0.88 ± 0.17
(D)	2.6 ± 21.9[b]	10.7 ± 0.8[b]	148 ± 8	71.2 ± 4.3[b]	6.8 ± 0.31[b]	21.2 ± 5.5	1.07 ± 0.42
(L)	41 ± 8.8[b]	10.2 ± 0.4	144 ± 11	83.2 ± 14.2	7.0 ± 0.48[b]	19.8 ± 2.7	0.80 ± 0.17
(I)	0.4 ± 27.5[b]	12.3 ± 0.8[b]	144 ± 9	81.6 ± 13.8	6.9 ± 0.25[b]	17.1 ± 2.4	1.30 ± 0.33
(H)	69 ± 12.9	13.7 ± 1.3	148 ± 8	99.6 ± 9.2	7.3 ± 0.27	21.7 ± 2.6	2.70 ± 1.12[b]
Female Group							
Control	7.0 ± 1	7.7 ± 0.3	174 ± 16	73.4 ± 5.7	6.6 ± 0.31	16.6 ± 1.3	1.00 ± 0.20
Outside Control	13.6 ± 5	8.1 ± 1.0	178 ± 7	68.3 ± 2.8	6.5 ± 0.13	19.4 ± 3.7	0.72 ± 0.23
(D)	-2.2 ± 6[b]	8.2 ± 0.9	152 ± 17[b]	61.0 ± 6.7	6.2 ± 0.18	15.4 ± 0.9[b]	0.50 ± 0.22
(L)	1.6 ± 11[b]	7.9 ± 0.3	166 ± 14	69.0 ± 2.2	6.4 ± 0.37	18.3 ± 2.6	0.80 ± 0.21
(I)	-1.2 ± 17[b]	9.2 ± 0.6[b]	154 ± 13[b]	72.0 ± 11.9	6.5 ± 0.29	14.9 ± 2.3[b]	0.78 ± 0.36
(H)	8.4 ± 13	9.7 ± 1.1[b]	171 ± 10	75.5 ± 6.6	6.6 ± 0.21	15.6 ± 1.9[b]	1.34 ± 0.90

a: values indicate a mean ± S.D. of five animals/sex/group. (L), (I), (H), and (D) are as described in Materials and Methods

b: significantly different from control ($P < 0.05$)

Table 2 Hematological Parameters in Rats Dosed with CLP's at 1000 mg/kg[a]

Group	Hgb g/100 ml	Hct (%)	RBC x $10^6/\mu l$	Poly[c] (%)	WBC x $10^3/\mu l$	Lymphocytes (%)
Male						
Control	14.8 ± 0.55	43.0 ± 2.1	7.5 ± 0.35	11.4 ± 6.2	8.2 ± 0.8	81.8 ± 5.2
Outside Control	14.6 ± 0.50	42.0 ± 2.4	7.2 ± 0.35	18.0 ± 5.7	9.7 ± 1.1	73.8 ± 5.1
(D)	12.2 ± 0.65[b]	34.9 ± 1.5[b]	6.1 ± 0.52[b]	50.0 ± 11.8[b]	24.0 ± 9.4[b]	39.8 ± 10.4[b]
(L)	13.8 ± 0.72	39.8 ± 2.4	7.0 ± 0.41	28.6 ± 9.8[b]	17.4 ± 9.7	62.0 ± 5.7[b]
(I)	13.1 ± 0.52[b]	37.5 ± 1.4[b]	6.6 ± 0.22	29.2 ± 13.9[b]	19.8 ± 4.9[b]	61.8 ± 13.5[b]
(H)	13.3 ± 0.47[b]	37.6 ± 1.6[b]	6.6 ± 0.36	16.8 ± 10.4	9.6 ± 2.0	77.0 ± 11.4
Female						
Control	13.9 ± 0.32	38.6 ± 1.2	6.4 ± 0.25	11.6 ± 5.2	9.7 ± 2.1	81.6 ± 7.0
Outside Control	13.8 ± 0.36	38.7 ± 1.3	6.8 ± 0.41	14.6 ± 7.9	7.0 ± 2.7	79.6 ± 8.3
(D)	12.3 ± 0.58[b]	34.5 ± 1.9[b]	5.9 ± 0.38[b]	42.4 ± 13.6[b]	15.6 ± 9.3	51.8 ± 13.8[b]
(L)	12.7 ± 0.62[b]	35.8 ± 1.4[b]	6.3 ± 0.28[b]	29.4 ± 10.0[b]	8.8 ± 2.3	63.6 ± 11.7[b]
(I)	11.6 ± 0.40[b]	32.8 ± 1.0[b]	5.6 ± 0.12[b]	39.0 ± 13.4[b]	10.2 ± 3.4	52.0 ± 11.6[b]
(H)	12.6 ± 0.46[b]	35.4 ± 1.6[b]	6.2 ± 0.31[b]	25.8 ± 10.4	8.4 ± 2.5	65.2 ± 10.1[b]

a: values indicate mean ± S.D. of five animals /sex/group. (L), (I), (H) and (D) are as described in Materials and Methods

b: significantly different from control ($P < 0.05$)

c: Abbreviation: Poly: polynucleated nentrophils

microsomes. This effect was previously reported and may be good indicator of exposure to heavier fractions of CLP's (Bostick et al 1981). There is no apparent correlation between biochemical findings and pathological finding in this study. However, in previous inhalation studies using (H) from an SRC-II process (Springer et al, 1986), exposure was found to affect body weights, hematology, and serum cholesterol and albumin. These investigators concluded that bone marrow is a target for (H). We can extend this argument to fractions (L) and (I). The hematological findings (i.e. rise in WBC) are associated with the induction of skin lesions and those findings of dyserythropoesis confirm that the CLP's have as a target organ the bone marrow. Diesel fuel used as a positive control exhibited the same effects as did (I), a result which is not surprising given they both have similar boiling points; perhaps similar hydrocarbon components.

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