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TOXICOLOGIC STUDIES OF EMISSIONS FROM A COAL GASIFICATION PROCESS — A CHRONIC FEEDING STUDY

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The health effects of the gasifier ash (0.5, 1.0 and 5.0% added to the diet) from a Lurgi coal gasification plant in Yugoslavia were studied in a chronic experiment in rats. The exposure lasted 32 weeks. The growth rate was lower only in animals exposed to the highest level of ash additive (5%). Determination of health effect parameters at the end of exposure i.e. of urinary protein excretion, hematological and histopathological examination revealed no morphological or functional impairment attributable to the treatment with ash. Therefore no health hazard of ash from coal gasification in conditions of environmental exposure is expected.

Coal gasification as a conversion process of coal usage will replace many of the present conventional processes in the near future (1). However, there are many uncertainties about the effect of this and related technologies upon man and his environment. The ash (slag) from coal gasification as the unburnable coal residue is considered to be the main single waste from such a process (2). This ash is a complex mixture of inorganic substances (3). The health effects of such mixtures are poorly understood, and so far the possible effects are predicted mainly on the bassis of their chemical composition (4, 5). Therefore we performed a series of experiments on rats in order to evaluate the possible health effects of the ash from a Lurgi coal gasification plant at Kosovo near Priština in Yugoslavia. Our previous subchronic experiment showed that the ash is not likely to alter some health effect parameters (6) although it contains many toxic heavy metals. In this paper we report the results

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of a longer exposure to ash i.e. the results of our chronic feeding study in which we exposed the animals during 32 weeks. No similar data are available in the literature. Our results suggest that presumably gasifier ash will not represent a serious health hazard in concentrations much higher than expected in the environment even in conditions of long-term exposure.

MATERIALS AND METHODS

Animals and treatment

In the experiment the random bred albino rats from the Institute's breeding farm were used. A total of 80 animals of both sexes (40 males and 40 females) entered the experiment at the age of six weeks. The control and each of the three ash treated groups consisted of 10 male and 10 female rats. The animals were kept in standard plastic cages, 10 animals per cage. The exposure period lasted 32 weeks. Individual body weights were recorded seven times over the experimental period. Experimental animals were fed diets with three different levels of gasifier ash i.e. 0.5, 1 or 5% of ground ash added to the powdered control diet (Pharmaceutical Works »Pliva«, Zagreb). Ash analysis showed the following macro and micro-components per 100 g of ash: Ca, Si, Fe, Mg, Al, Na, K, Ti, S, P and Mn 0.1 — 30 g and Cr, Cu, Zn, Pb, and Cd 0.1 — 9 mg, respectively. All diets pelletted in the same way and deionised water were offered to animals *ad libitum*. The nutritional composition of the control diet, the analytical data of the ash and a detailed description of the method used for sampling and treatment of ash were reported earlier (6, 7, 8).

Determination of health effect parameters

Urinary protein excretion. At the end of the 32-week-exposure period urinary protein excretion was determined in seven male end seven female rats from each group. The same animals were used for hematological examination. The rats were placed in individual metabolic cages for two days. During the second day 22-hour urine samples were collected at room temperature. Proteins were determined according to *Piscator* (9) on a Unicam SP-500 spectrophotometer at the 330 nm wavelength. The results were expressed as micrograms of protein in 1 hour per 100 cm^2 body area to allow comparison of animals with different body weights and kidney mass (10).

Hematological examination. Blood samples were taken from the tip of the tail. Leucocyte and erythrocyte counts were performed with standard diluting pipettes and counting chambers. Packed cell volume (PCV) was determined by the microhematocrit method. Hemoglobin concentration was determined by the standard acid hematin method on a

Unicam SP-600 spectrophotometer at the 540 nm wavelength. Mean corpuscular volume (MCV), mean corpuscular hemoglobin level (MCH), and mean corpuscular hemoglobin concentration (MCHC) were calculated.

Histopathological examination. At the end of the experiment necropsy was performed in all animals after they had been exsanguinated in excess of ether. Gross pathology observations were made on all organs of the thoracic and abdominal cavities. Wet weights of the liver and kidneys, and of the testicles in males, were determined in seven animals of each sex, from each group. Histological analysis of the liver and kidneys, and of the testicles in males, was performed in HE stained paraffin preparations using light microscopy in three animals of each sex from each group.

RESULTS

Thorough daily monitoring showed that all animals were in good health throughout the experiment. From the body weights presented in Table 1 a growth retardation was observed in animals exposed to the highest ash level (5%). This effect was more pronounced in males (on the average about 20% lower values compared with controls) than in females (on the average about 11% lower values).

Table 1.

Body weights of rats (g) fed diets with three levels of ash additive. Results are presented as arithmetic means ± SEM of ten animals of each sex in each group.

Sex	Length of exposure (weeks)	Controls	Ash additive to diet (%)		
			0.5	1.0	5.0
Males	0	129.9±2.5	—	—	—
	4	252.0±4.7	220.5±4.6	249.5±3.1	197.5±7.9
	8	327.5±6.3	263.5±6.3	287.5±5.2	217.5±5.1
	16	297.0±5.0	297.5±5.4	318.5±5.3	269.5±7.0
	20	357.5±7.6	329.0±5.7	350.5±5.9	300.5±8.4
	24	369.0±7.4	343.5±7.1	366.5±5.8	308.0±7.5
	32	370.5±9.2	353.5±5.7	373.0±5.2	297.0±7.3
Females	0	114.8±2.1	—	—	—
	4	175.0±5.7	169.0±6.1	169.0±2.3	150.5±3.9
	8	196.5±3.7	190.0±5.9	187.0±1.7	178.0±4.7
	16	216.5±4.5	203.0±7.3	207.0±2.9	192.5±4.8
	20	226.5±5.5	210.5±6.9	211.0±2.5	199.5±5.0
	24	231.0±3.6	214.0±7.1	217.5±2.8	208.5±6.3
	32	235.5±4.6	227.0±7.7	230.0±3.4	216.0±8.7

Organ weights presented as percentage body weight in Table 2 showed constant values irrespective of the dietary treatment with ash, indicating that changes in body weight were always associated with proportional changes in organ weight.

Table 2.

Relative organ weights in rats exposed to diets with three levels of ash additive during 32 weeks. Values of 7 male and 7 female rats in each group are presented as arithmetic means ±SEM.

Sex	Ash additive to diet (%)	Weight as percentage of body weight of		
		kidneys	liver	testicles
Males	0	0.63±0.02	3.35±0.09	0.75±0.03
	0.5	0.61±0.02	3.04±0.12	0.70±0.04
	1.0	0.61±0.03	2.99±0.10	0.73±0.02
	5.0	0.63±0.02	3.31±0.16	0.84±0.01
Females	0	0.71±0.02	3.77±0.15	—
	0.5	0.69±0.02	3.90±0.18	—
	1.0	0.67±0.01	3.38±0.08	—
	5.0	0.65±0.02	3.79±0.11	—

The excretion of proteins via urine presented in Table 3 was not affected by the dietary treatment with different levels of ash additive. Sex related differences are in agreement with our previous results and findings of other authors (6, 10).

Table 3.

Urinary proteins in rats exposed to diets with three levels of ash additive during 32 weeks. Results are presented as arithmetic means ±SEM of 7 animals of each sex in each group. Urine was collected during 22 hours.

Ash additive to diet (%)	μg of proteins/h/100 cm² body area	
	Males	Females
0	141±7	74±5
0.5	128±9	74±6
1.0	138±10	73±7
5.0	100±8	69±9

Blood values presented in Table 4 did not indicate any influence of the treatment with ash. Again sex differences were noticed, so all the measured parameters (leucocyte and erythrocyte counts, PCV and he-

Table 4.

Hematological parameters in rats exposed to diets with three levels of ash additive during 32 weeks. Results are presented as arithmetic means ±SEM of 7 animals of each sex in each group.

Sex	Ash additive to diet (%)	Leucocytes (10^3 μl)	Erythrocytes (10^6 μl)	PCV (%)	Hemoglobin	MCV (μ^3)	MCH (pg)	MCHC (%)
Males	0	13.1±0.7	7.6±0.1	51.9±0.5	14.7±0.2	68.5±0.9	19.6±0.2	28.3±0.3
	0.5	12.8±0.8	7.7±0.2	48.4±0.7	14.1±0.2	63.7±1.6	18.5±0.5	29.2±0.3
	1.0	12.8±0.7	7.1±0.1	48.6±0.7	13.7±0.1	69.1±0.9	19.4±0.3	28.2±0.3
	5.0	13.6±1.1	7.2±0.1	49.8±0.7	13.8±0.3	69.7±1.2	19.3±0.5	27.8±0.6
Females	0	9.4±0.8	7.1±0.1	48.5±0.5	13.6±0.1	68.5±1.3	19.8±0.3	28.1±0.3
	0.5	11.0±0.9	6.8±0.2	47.5±0.6	13.9±0.3	70.0±1.7	20.4±0.3	29.3±0.5
	1.0	8.1±0.9	6.6±0.1	46.4±0.3	13.2±0.2	70.9±1.2	20.0±0.4	28.4±0.4
	5.0	10.8±0.3	6.4±0.2	46.7±0.7	13.6±0.1	73.7±1.7	21.4±0.6	29.2±0.4

moglobin were slightly higher in males, whereas the calculated parameters (MCV and MCH) were slightly higher in females. These findings are also in agreement with our previous results (6).

Gross pathology and a histological examination of the liver, kidneys and testicles confirmed our other negative results because no differences between control and exposed animals were found either in males or in females.

DISCUSSION

The results of the present chronic study and the results of our previous subchronic feeding study in which animals were exposed to different levels of ash additive during 16 weeks (6) are essentially the same. The health effect parameters determined in exposed animals did not differ from controls irrespective of the level of exposure. The only consequence of exposure to ash in diet was shown in animals exposed to the highest level i.e. 5%. In these animals a growth retardation, more pronounced in males than in females, was recorded. It was described earlier that this effect was even more pronounced in weanlings, being associated with reduced food intake (19% in females and 27% in males) but reversibile after the animals had returned to control diet (11). Changes in body weights were always associated with proportional changes in organ weights. Therefore no selective effects on organ growth are likely to occur. On the basis of our results we assume that very high concentrations of ash in diet represent a »nutritional stress«, which affects animals in the phase of rapid growth, especially weanling males, rather than a toxic action attributable to some components of ash. The no-effect conclusion was also confirmed in our three-generation reproduction study which did not reveal any mutagenic or teratogenic potential of ash (8).

There are several possible explanations why ash as a complex mixture of both toxic and essential inorganic substances produced no measurable health effects in our experiments. One of the assumptions is that the elements present in ash assumed the chemical form characterized by poor solubility and therefore were not biologically available. The low leachability of ash (12, 13) and some of our pharmacokinetic data support this hypothesis (14, 15). Further, high dietary levels of some essential elements like calcium and iron could influence the gastrointestinal absorption of toxic elements and thus prevent their toxic effects. It should be also mentioned that in our experiments the daily dietary intake from the ash of the most hazardous elements like Cd, Pb and As (25.5, 51.9 and 47.6 μg/animal respectively) was still below the levels known to influence the health effect parameters determined (16). Lastly, the health effect parameters might not have been sensitive enough to detect slight changes possibly induced by ash. Also, the exposure time

might have been too short to determine a potential carcinogenic risk. It can be concluded only that at present there is no evidence of toxic effects of ash from coal gasification in experimental animals.

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Sažetak

TOKSIKOLOSKA ISTRAŽIVANJA EFLUENATA IZ PROCESA UPLINJAVANJA UGLJENA. KRONIČNI POKUS

Svrha ovog rada je bila ispitivanje mogućih učinaka šljake iz procesa rasplinjavanja ugljena na neke pokazatelje zdravstvenog stanja u štakora. Pokusne životinje su bile izložene šljaci putem hrane. Miješanjem samljevene šljake i standardne smjese za štakorsku hranu pripremljene su hrane koje su sadržavale 0,5 1,0 i 5,0% šljake. Štakori obaju spolova, koji su na početku pokusa bili stari 6 tjedana, bili su izloženi šljaci tokom 32 tjedna. Za cijelo vrijeme trajanja pokusa životinje su svakodnevno promatrane radi praćenja mogućih promjena zdravstvenog stanja, a sedam puta tokom pokusa su svim životinjama izmjerene tjelesne težine. Na kraju pokusa određena je koncentracija proteina u mokraći, te standardna krvna slika. Sve životinje su nakon žrtvovanja u suvišku etera makroskopski pregledane, a u nekih je životinja učinjen i histološki pregled organa. Rezultati ovog kroničnog pokusa su pokazali da tretman šljakom nije utjecao na promatrane pokazatelje zdravstvenog stanja. Jedino je u životinja izloženih najvišoj koncentraciji šljake u hrani (5%) opaženo zaostajanje u rastu koje je bilo nešto izraženije u mužjaka. Na temelju rezultata prikazanih u ovom radu kao i naših ranijih istraživanja toksičnosti šljake i njezinih ekstrakata može se pretpostaviti da šljaka, iako sadržava niz toksičnih elemenata, neće predstavljati ozbiljnu opasnost za zdravlje kada se nađe u okolišu, čak ni u uvjetima kronične izloženosti viših organizama.

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