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# REPRODUCTIVE TOXICOLOGICAL EFFECTS IN RATS AFTER ORAL EXPOSURE TO EFFLUENTS FROM A COAL GASIFICATION PLANT

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Health effects of three liquid wastes from a Lurgi coal gasification plant were studied in rats. Fleissner condensate (F), Generator section waste water (G) and Phenosolvan section waste water (P) were given to rats as drinking water through three generations to determine some reproduction parameters (fertility index, number of pups per litter and body weight). Other health effect parameters (body weight, food and fluid consumption, trace element concentration in the body, haematological values, femur morphometry data, gross necropsy and histopathology findings) were recorded in $F_1$, $F_2$ or $F_3$ generation after different lengths of postnatal exposure (6 to 12 months).

Results* show that the G and F effluents did not cause any specific health effect in rats, whereas the P effluent caused lower daily fluid consumption, decreased body weight at all stages of development, increased copper concentration in the carcass, smaller bones, atrophic organs and haemosiderosis in the liver. These changes might be related to the phenol content of this effluent.

The environment of a coal gasification plant is contaminated with various gaseous, solid, and liquid waste products. The Lurgi coal gasification plant situated in the south of Yugoslavia is one of the biggest plants of this type in this part of Europe. A considerable amount of solid and liquid waste is being deposited on the ground or into the river near the plant. Therefore, the environment surrounding the plant is permanently exposed to inorganic elements (essential and toxic) or organic pollutants which enter the biological cycle.

Results, which have accumulated in recent years, indicate that chronic oral exposure to gasifier ash (1) or gasifier ash leachates (2, 3) from the coal gasification process has practically no effect on several health parameters in rats.

* Some of these results were reported to the Third Congress of Croatian Biological Society with international participation, Mali Lošinj, 1987.

The purpose of our present experiments was to evaluate the health effects of some liquid wastes produced by the same Lurgi coal gasification plant which enter into the environment. Therefore a three-generation reproduction study was performed on rats. Reproduction parameters and additional health effects were determined in rats of the $F_1$, $F_2$ and $F_3$ generations.

## EXPERIMENTAL

### *Effluents*

The effluents administered to the animals were: Fleissner condensate (F), Generator section waste water (G) and Phenosolvan section waste water (P).

*Fleissner condensate (F)* is a liquid waste produced in the process of coal drying. Before it is used in the gasification process lignite is dried by the »Fleissner process« down to the moisture content of approximately 25 per cent. This condensate is transported through pipe lines to the same place as gasifier ash and deposited on ground near to the plant.

*Generator section waste water (G)* contains suspended particles of coal and ash, because it originates from expander cyclones. This water is discharged into the river near the plant.

*Phenosolvan waste water (P)* is obtained after the process of partial extraction of phenols in the Phenosolvan section before final biological treatment. It is also discharged into the same river.

The above mentioned effluents were collected and transferred in plastic containers to the Institute for Medical Research and Occupational Health in Zagreb. The pH of the F and G effluents was about 7, and that of the P effluent about 11. The effluents were analysed for inorganic elements by Spark Source Mass Spectrometry (SSMS) (Table 1). All effluents had similar inorganic composition. The elements can be classified into three groups. The range of Cl and S concentrations in all effluents was between 100 and 1000 ppm. The elements Na, K, Mg, Ca, Ba, Fe, P, Si, F and Br were between 1 and 100 ppm. Trace elements, mostly toxic heavy metals (I, Cd, Mo, As, Zn, Cu, Ni, Cr, V, B, Be), were below 1 ppm.

Organic components were also analysed by means of total chromatographable organics, by gravimetric analysis (4) and by gas chromatography-mass spectrometry. The Phenosolvan section waste water had the greatest total organic concentration of the three waste waters. Its organic content of 0.13 mg/ml was approximately three times higher than the total organic concentration of the Fleissner condensate (0.04 mg/ml) and 52 times higher than the total organic concentration of the Generator section water (0.0025 mg/ml). Approximately 45 per cent of the Phenosolvan organics and only 7 per cent of the Fleissner condensate organics were identified. The most significant compound was found to be phenol. Its concentration was 0.038 mg/ml in Phenosolvan waste water. In Fleissner condensate phenol was the only identified organic constituent (0.0027 mg/ml). The Generator section waste water did not have any peaks large enough to be identified and was essentially free of organics.

Table 1.

*Concentration of some inorganic elements in the F, G and P effluents*

| Element | μg/ml in original effluents | | |
|---|---|---|---|
| | F | G | P |
| Ba | 0.78 | 1.6 | 1.8 |
| I | 0.94 | 0.24 | 0.94 |
| Cd | 0.98 | 0.48 | 0.98 |
| Mo | < | < | 0.94 |
| Br | 10 | 2.8 | 2.8 |
| As | < | < | 0.22 |
| Zn | 2.8 | 0.38 | 0.74 |
| Cu | 0.62 | 0.30 | 0.13 |
| Ni | 1.3 | 0.64 | 0.64 |
| Fe | 7.4 | 30 | 7.4 |
| Cr | 0.22 | 0.20 | 0.94 |
| V | 0.038 | 0.040 | 0.24 |
| Ca | 24 | 50 | 6.0 |
| K | 6.8 | 3.2 | 6.8 |
| Cl | 180 | 88 | 180 |
| S | 780 | 190 | 780 |
| P | 1.3 | 1.4 | 1.4 |
| Si | 40 | 17 | 20 |
| Mg | 16 | 0.80 | 3.8 |
| Na | 200 | 14 | 30 |
| F | 40 | 9.8 | 2.4 |
| B | 0.032 | 0.48 | 1.0 |
| Be | < | 0.024 | 0.026 |

Results were obtained by SSMS analysis (Spark Source Mass Spectrometry), performed by R. Merrill, Environmental Protection Agency, Research Triangle Park, USA.

F — Fleissner condensate
G — Generator section waste water
P — Phenosolvan waste water
< below detection limit

## MATERIALS AND METHODS

### *Experimental design*

The experiment was performed as a three-generation reproduction study. It started with 240 albino rats (120 males and 120 females) from the Institute's breeding farm (parental $F_0$ animals). At the beginning of experiment the males and females were 18 weeks old and weighed about 280 and 210 grams, respectively. Exposed rats were divided randomly into four groups of 30 animals each (10 animals per cage). Three experimental groups received the F, G or P effluent as drinking water, and the control group (C) received tap water. Dosing was continuous throughout three generations. All animals were given the same standard rat diet (»Sljeme«, Zagreb; 1.2% Ca and 0.8% P). Food and drinking

water were supplied to all groups *ad libitum*. Individual female and male rats were mated in Macralon cages for three days. Reproduction parameters (fertility index, number of pups per litter and body weight) were recorded in mothers and sucklings up to 14 days after birth. Other health effect parameters (body weight, food consumption, trace element concentration, haematological values, femur morphometry data, gross necropsy and histopathology findings) were recorded in randomly chosen rats of the $F_1$, $F_2$ or $F_3$ generation after various lengths of postnatal exposure. The $F_1$ and $F_2$ generations were given to effluents as drinking water for six months after birth, and the $F_3$ generation was so exposed for 12 months.

*Reproduction parameters*

After mating of the $F_0$ animals, pregnant females were caged individually. Deliveries were recorded each morning up to the 26th day. The whole litter was weighed 24 hours later and the mean individual body weight of the pups was calculated. The same day the litter size was adjusted to six animals, without reference to sex. The body weights of mothers, fertility index and number of pups per litter were recorded and the same measurements were repeated on the 14th day after delivery when some pups from each group were killed for chemical analysis. The rest of the sucklings were weaned at 21st day of age, separated by sex, placed into plastic cages in groups of about 10 animals per cage, and exposure to the effluents continued. Individual males and females of the $F_1$ generation were mated at the age of 12 weeks at the same 1 : 1 basis. One male and one female rat were randomly selected from each litter for cross mating with pups from another litter of the same group (F, G, P or C) to produce the $F_2$ generation. The same mating procedure was repeated with the $F_2$ generation to obtain the $F_3$ generation. The reproduction effect was evaluated when the third generation of litters were 14 days old.

*Other health effect parameters*

*Body weight and food consumption.* Body weight was recorded for each animal at two-weekly intervals. Food and water consumption was measured for four consecutive days each month during the exposure period and the means of these four-day values expressed per animal and per day.

*Trace element analysis.* Some 14-day-old rats from the $F_1$ and $F_3$ generations were anaesthetized in ether and exanguinated from the abdominal aorta for trace element analysis. Their carcasses (whole body without liver, kidney and gut) were weighed and dry ashed at 450 °C. The ash was dissolved in 10 per cent nitric acid, adjusted to 10 ml and the trace elements were measured by flame atomic absorption spectrophotometry (Varian Instrument, AA-375).

*Haematological examination.* Blood samples from the tip of the tail were taken from males of $F_1$ generation after six months of exposure. Leucocyte and erythrocyte counts were made with standard diluting pipettes and counting chambers. Packed cell volume (PCV) was determined by the microhaematocrit method using heparinized microhaematocrit tubes, centrifuged at 12000 rpm for two minutes. Haemoglobin concentration was determined by the

standard acid haematin method (with haemoglobin cyanide solution as standard — International Reference, RIJKS Institute, Bilthoven, The Netherlands) on a Unicam SP-600 spectrophotometer at 540 nm wavelength and the mean corpuscular volume (MCV), mean corpuscular haemoglobin level (MCH) and mean corpuscular haemoglobin concentration (MCHC) were calculated (5).

*Morphometric bone measurements.* For morphometric measurements 10 male animals from the $F_2$ generation were used. Right femurs were dissected and contact X-rays made. Femur length (L), total midshaft (T) and medullary midshaft width (M) were measured under magnification. From these measurements, total area (TA), medullar area (MA), cortical area (CA) and cortical index (CA/TA) were calculated according to *Garn and co-workers* (6). The same femurs were then dry ashed at 450 °C, the weight of ash was determined and expressed as ash per unit femur length.

*Histopathological examination.* In all animals necropsy was performed after the rats were exanguinated from the abdominal aorta. Gross necropsy observations were made in all organs. Histopathological analysis of organs was performed on five 14-day-old pups from the $F_3$ generation and in five adult males and females from each group in the same generation after 12 months of postnatal exposure. Paraffin sections of the stomach, liver, kidneys and testicles were stained with haematoxylin-eozin for light microscopic examination. In some liver samples histochemical reaction with Prussian blue was made for the determination of deposited haemosiderin.

*Statistical treatment.* Student's t-test was used for evaluation of results. Each exposed group was compared with the control non-exposed group. Some results (fertility index) are presented as percentages of the control values.

## RESULTS

### *Three-generation reproduction parameters*

The incidence of pregnancy in females from three subsequent generations exposed to the F, G and P effluents (53—83%) was within the normal range of pregnancies in control animals (55—78%). No difference was observed in the number of animals per litter between control ($\bar{x} = 8.9 \pm 0.5$, range 3—13) and exposed groups ($\bar{x} = 9.3 \pm 0.5$, range 2—13) in any generation.

The body weights of the newborn and fourteen-day-old animals in the group exposed to the F and G effluents showed no difference compared to controls. However, body weights of animals from the groups of the $F_1$ and $F_2$ generations exposed to the P effluent were significantly lower ($P < 0.01$) than in control groups (Table 2). The same difference was observed in the $F_3$ generation animals exposed to the P effluent but only at the age of fourteen days. Body weights of female rats from three subsequent generations (Table 3) during the lactation period (on the first and fourteenth day after delivery) were significantly lower ($P < 0.001$) than in their controls, only in groups exposed to the P effluent.

Table 2.

*Body weight and number of sucklings in three subsequent generations*

| Groups | No. of animals | Weight of newborns (g)[a] | Weight at the age of 14 days (g) |
|---|---|---|---|
| | | $F_1$ generation | |
| C | 95 | 5.8 ± 0.2 | 22.8 ± 0.3 |
| F | 81 | 5.7 0.2 | 21.0 0.6 |
| G | 115 | 5.8 0.1 | 23.2 0.3 |
| P | 97 | 5.1 0.1** | 18.0 0.3*** |
| | | $F_2$ generation | |
| C | 135 | 6.2 ± 0.1 | 26.8 ± 0.3 |
| F | 120 | 6.1 0.1 | 26.6 0.3 |
| G | 132 | 5.9 0.1 | 28.1 0.2 |
| P | 108 | 5.7 0.1** | 23.1 0.3*** |
| | | $F_3$ generation | |
| C | 103 | 5.8 ± 0.2 | 23.8 ± 0.4 |
| F | 138 | 5.8 0.1 | 24.6 0.2 |
| G | 120 | 6.0 0.1 | 26.2 0.3 |
| P | 96 | 6.1 0.1 | 22.5 0.3** |

Results are presented as arithmetic means ± SEM.

[a]Parameters taken 24 hours after birth

C — control; F — Fleissner condensate; G — Generator section waste water; P — Phenosolvan section waste water.

** $P < 0.01$; *** $P < 0.001$.

*Other health effect parameters*

The general appearance of rats in all groups was good and no clinical signs of toxicity or morphological abnormalites were noticed. Morbidity and mortality were less than 2 per cent for the experiment irrespective of treatment.

Daily food consumption for adult males was about 24 grams and for females about 17 grams. No difference in daily food consumption between control and exposed animals was noted. Daily fluid intake in conditions of C or F and G effluent consumption was approximately 31—39 ml for males and about 26—28 ml for females. No difference was observed between controls and animals exposed to the F and G effluents. However, in animals of both sexes exposed to the P effluent in all generations a statistically significant reduction of effluent intake of about 40 per cent was noticed ($P < 0.001$) i.e. 22.5 ml in males and 14.7 ml in females.

No difference was observed in body weights between control animals (C) and animals of either sex exposed to the F and G effluents. In rats exposed to

Table 3.

*Effect of F, G, or P effluents on the body weight of female rats from three subsequent generations during the lactation period*

| Generation | Group | Body weight (g) | | | | | |
|---|---|---|---|---|---|---|---|
| | | 1st day after delivery | | | 14th day after delivery | | |
| First | C | 237.6 | ± 3.9 | (19) | 254.1 | ± 4.3 | (16) |
| | F | 241.6 | 3.2 | (16) | 251.3 | 2.9 | (15) |
| | G | 238.3 | 5.3 | (21) | 255.8 | 4.6 | (20) |
| | P | 206.3 | 4.5 | (23)*** | 220.5 | 4.0 | (20)*** |
| Second | C | 237.4 | ± 3.9 | (23) | 251.1 | ± 5.1 | (23) |
| | F | 238.1 | 4.2 | (21) | 246.0 | 4.2 | (20) |
| | G | 236.4 | 4.1 | (22) | 251.8 | 3.3 | (22) |
| | P | 194.2 | 4.1 | (18)*** | 196.4 | 5.2 | (18)*** |
| Third | C | 225.6 | ± 4.1 | (18) | 252.8 | ± 7.0 | (18) |
| | F | 230.4 | 4.3 | (23) | 253.3 | 4.7 | (23) |
| | G | 224.3 | 3.5 | (20) | 240.3 | 5.2 | (20) |
| | P | 207.1 | 3.8 | (17)*** | 210.0 | 4.5 | (16)*** |

Results are presented as arithmetic means ± SEM. Number of animals in parentheses.

C — control; F — Fleissner condensate; G — Generator section waste water; P — Phenosolvan section waste water.

*** $P < 0.001$.

the P effluent body weights were statistically lower than in the control group ($P < 0.001$) at all intervals. This is shown in Table 4 for the $F_3$ generation of animals.

Concentrations of some essential elements in the carcasses of fourteen-day old animals from the $F_1$ and $F_3$ generations are presented in Table 5. The two generations showed no difference in iron and zinc concentrations. However, copper concentrations in the carcasses of animals from both generations exposed to the P effluent were significantly higher ($P < 0.001$) than in control animals. There were no differences between the F and G effluent groups and controls.

Haematological parameters measured in the $F_1$ generation males six months after postnatal exposure did not practically differ between control animals and those exposed to the F, G or P effluent (Table 6).

Morphometric measurements of the femur in male rats from the $F_2$ generation (Table 7) showed significantly lower values ($P < 0.001$) of almost all parameters (femur length, ash/unit length, total area, medullar area and cortical area) in animals exposed to the P effluent. However, the cortical index was unchanged. Animals exposed to other effluents (F and G) did not differ from the controls in any morphometric parameter.

Histopathological findings in all organs were normal except in animals exposed to the P effluent. In sucklings changes were observed in the liver. The

Table 4.

*Body weight of rats ($F_3$ generation) during postnatal exposure to effluents*

| Group | Age (weeks) | | | | | |
|---|---|---|---|---|---|---|
| | 4 | 12 | 20 | 28 | 36 | 44 |
| | | | Males | | | |
| C | 94 (2) | 287 ( 7) | 351 ( 6) | 372 ( 8) | 352 ( 8) | 391 (10) |
| F | 98 (2) | 289 ( 6) | 353 ( 6) | 381 ( 6) | 361 ( 7) | 383 ( 6) |
| G | 101 (3) | 295 (12) | 363 (10) | 394 (10) | 394 (11) | 414 (15) |
| P | 93 (2) | 255 ( 5)*** | 322 ( 8)*** | 323 ( 9)*** | 315 (10)*** | 326 1(0) |
| | | | Females | | | |
| C | 86 (2) | 198 ( 4) | 229 ( 5) | 243 ( 4) | 237 ( 5) | 260 ( 6) |
| F | 82 (1) | 199 ( 4) | 228 ( 5) | 241 ( 5) | 237 ( 6) | 256 ( 5) |
| G | 93 (2) | 198 ( 4) | 223 ( 5) | 237 ( 5) | 240 ( 6) | 254 ( 9) |
| P | 77 (1) | 183 ( 3)*** | 202 ( 4)*** | 210 ( 6)*** | 209 ( 4)*** | 229 ( 6) |

Results are presented as arithmetic means of 9—20 animals in a group, SEM in parentheses.
C — controls; F — Fleissner condensate; G — Generator section waste water; P — Phenosolvan section waste water.
*** $P < 0.001$

number of Kuppfer cells was slightly increased and they had hyperchromatic nuclei without signs of phagocytosis. In adult rats the same changes were observed in the liver but in some animals deposited haemosiderin (positive histochemical reaction with Prussian blue) was also noticed. Atrophic changes in organs — observed at necropsy were also evident in histological preparations.

## DISCUSSION

The toxicity of a mixture of chemicals (organic and inorganic) occurring in various effluents or wastes is difficult to evaluate since very few data are available for specific mixtures. One way of determining mixture toxicity is to use data for its individual chemical components and to apply the »additivity« approach i.e. to sum up the individual toxicities. The additivity approach, however, has several shortcomings. Sufficient data might not be available for individual components and also, it does not consider the potential mutual interaction of individual chemical compounds. The additivity approach can be used in the absence of quantitative information on the toxicity of »simple mixtures« i.e. mixtures containing only a few chemical components. It is practically impossible to apply this approach as a model in cases of »complex mixtures« i.e. mixtures containing several chemicals.

The best way of estimating the toxicity of a complex mixture is to test the mixture as it occurs in the environment (actual wastes or effluents) or to prepare a similar mixture of chemical compounds in the laboratory and to test its toxicity.

Table 5.

*Concentrations of iron, zinc and copper in carcasses of fourteen-day old animals*

| Group | μg per g wet tissue | |
|---|---|---|
| | $F_1$ generation | $F_3$ generation |
| | Iron | |
| C | 25.0 ± 0.4 (21.9—27.4) | 23.4 ± 0.4 (20.5—25.6) |
| F | 23.8 0.4 (21.6—25.9) | 22.5 0.6 (18.4—26.7) |
| G | 24.2 0.5 (21.2—27.4) | 21.9 0.9 (16.9—26.7) |
| P | 26.5 0.6 (22.9—29.8) | 23.7 0.7 (18.1—29.1) |
| | Zinc | |
| C | 27.6 ± 0.5 (25.2—30.7) | 26.6 ± 0.4 (23.6—27.8) |
| F | 26.8 0.7 (20.7—31.5) | 26.4 0.8 (18.7—32.2) |
| G | 26.3 0.6 (22.1—30.3) | 27.3 0.9 (20.3—37.0) |
| P | 26.9 0.6 (23.9—32.1) | 27.5 0.6 (19.4—30.4) |
| | Copper | |
| C | 1.91 ± 0.12 (1.36—2.81) | 1.67 ± 0.03 (1.46—1.84) |
| F | 1.78 0.09 (1.61—2.00) | 1.75 0.04 (1.59—2.09) |
| G | 1.75 0.04 (1.54—2.14) | 1.69 0.06 (1.31—2.20) |
| P | 2.68 0.13 (1.83—3.39)*** | 1.90 0.04 (1.58—2.19)*** |

Results are presented as arithmetic means of 15 animals in each group ± SEM (a range in parentheses)

C — control; F — Fleissner condensate; G — Generator section waste water; P — Phenosolvan section waste water.

Carcass = whole body without liver, kidney and gut

*** $P < 0.001$.

Table 6.

*Haematological parameters in adult male rats of the $F_1$ generation exposed for six months to the F, G or P effluent*

| | GROUPS | | | |
|---|---|---|---|---|
| | C | F | G | P |
| Leucocoytes ($\mu l^3$) | 15.2 ± 1.1 | 16.8 ± 0.5 | 16.1 ± 1.5 | 14.1 ± 0.8 |
| Erythrocytes ($\mu l^6$) | 7.8 ± 0.1 | 7.3 ± 0.3 | 7.0 ± 0.3 | 7.5 ± 0.1 |
| PCV (%) | 49.2 ± 0.6 | 49.8 ± 0.5 | 49.7 ± 0.6 | 49.3 ± 0.5 |
| Haemoglobin (%) | 14.7 ± 0.3 | 14.3 ± 0.2 | 14.9 ± 0.2 | 14.8 ± 0.3 |
| MCV ($\mu m^3$) | 63.7 ± 1.8 | 68.9 ± 2.5 | 71.2 ±2.2 | 66.3 ± 1.5 |
| MCH (pg) | 19.0 ± 0.5 | 19.8 ± 0.7 | 21.3 ± 0.6 | 19.9 ± 0.5 |
| MCHC (%) | 29.9 ±0.4 | 28.8 ±0.4 | 30.0 ± 0.4 | 30.1 ± 0.3 |

Results are presented as arithmetic means of 10 animals in each group ± SEM. C — controls; F — Fleissner condensate; G — Generator section waste water; P — Phenosolvan section waste water.

Table 7.

*Morphometric parameters of the femur in the $F_2$ generation males six months after postnatal exposure to effluents*

| | Group | | | |
|---|---|---|---|---|
| | C | F | G | P |
| L (mm) | 39.82 ± 0.32 | 40.23 ± 0.16 | 40.38 ± 0.29 | 37.23 ± 0.36*** |
| Ash/L (mg/mm) | 13.84 ± 0.50 | 13.02 ± 0.20 | 13.56 ± 0.25 | 10.38 ± 0.21*** |
| TA ($mm^2$) | 17.38 ± 0.69 | 16.71 ± 0.90 | 17.52 ± 0.44 | 13.77 ± 0.32*** |
| MA ($mm^2$) | 6.43 ± 0.42 | 6.29 ± 0.29 | 6.08 ± 0.28 | 5.05 ± 0.30** |
| CA ($mm^2$) | 10.94 ± 0.55 | 10.41 ± 0.68 | 11.45 ± 0.31 | 8.73 ± 0.24** |
| CA/TA | 0.629 ± 0.018 | 0.617 ± 0.016 | 0.653 ± 0.012 | 0.634 ± 0.017 |

Results are presented as arithmetic means of 10 animals ± SEM. L — femur length; TA — total area; MA — medullar area; CA — cortical area; CA/TA — cortical bone index. C — controls; F — Fleissner condensate; G — Generator section waste water; P — Phenosolvan section waste water
**$P < 0.01$; ***$P < 0.001$.

In our experiment we estimated the toxicity of actual effluents from the coal gasification plant without any dilution since in preliminary experiments we found that rats maintained on effluents as drinking water survived the fourteen-day testing period. We used the three-generation reproduction studies as the best model for testing the toxicity of an unknown mixture to be able to make observations at all stages of development through several generations.

Our preliminary results show that two of the three effluents tested i.e. Generator section waste water and Fleissner condensate do not cause any specific health effects in rats. This statement is based on morbidity and mortality data, body weights, food and fluid consumption, reproduction parameters (fertility index, number and weight of newborn and fourteen-day old pups), trace element analysis (Zn, Fe, Cu), haematological values, bone morphometry parameters, necropsy and organ histology findings.

The third effluent — Phenosolvan unit waste water was found to cause decreased body weights at all stages of development. At the same time food consumption was not reduced. Only lower daily fluid consumption was observed. Another change observed was an increased copper concentration in the carcasses. Bone morphometry data showed shorter and smaller bones but no changes in bone density (cortical bone indices) in these animals. Histopathological changes included atrophic organs and slight changes in the liver including haemosiderosis. No changes compared to controls were observed in other health effect parameters (morbidity, mortality, haematological values) and reproduction indices (fertility index and number of pups).

We can assume that the concentration of inorganic elements in the P effluent was too low to cause these health effects since in our previous experiment in which rats had been exposed to gasifier ash or gasifier ash leachates no health effects were observed although the concentrations of the same inorganic elements were much higher (2, 3). Of organic components in the P efflu-

ent phenol alone was identified in larger quantities. Animals were actually exposed to about 2—4 mg phenol (2.3 mg females and 3.5 mg males) per kg body weight. Therefore, we tried to compare our findings within the known toxicity values for phenol.

Regardless of the mode of administration, phenol is known to readily gain access to body tissues. Absorbed phenol mainly conjugates with glucuronic and sulphuric acids (7). The extent of conjugative metabolism varies with dosage. Investigations of phenol distribution and metabolism in rats show that it undergoes a very large »first pass« effect when given orally. Only 3 per cent of the dose appears as the parent compound in the systemic circulation (8). At a low phenol dose (< 1 mg/kg) intestinal and hepatic conjugations are comparative. The capacity of intestinal conjugative enzymes is remarkably high; at large doses (> 5 mg/kg) intestinal conjugation exceeds by far the contribution of the hepatic and pulmonary enzymes (9). Therefore the highest level of phenol detoxication appears to occur in the digestive tract rather than in the liver (7). Phenol is toxic to the mammalian kidney. Some consider the renal effect to be secondary to the haematopoetic one although direct toxicity of phenol on the kidney has been proved (10). In our experiment kidney lesions were not observed. The haemosiderosis of the liver found in some animals points to haematopoetic effects although no changes in haematological parameters were observed. In chronic inhalation experiments with phenol some authors observed changes in the sexual cycle of female white rats at phenol concentrations of 5 and 0.5 mg/m³. Marked changes in the functional condition of the ovaries — shortening of the stage of oestrus and lengthening of the stage of inactivity — occurred at the higher dose level (11). Phenol in aqueous solution administered orally with a stomach tube caused dose related changes in chromosomal sets of spermatogonia and primary spermatocytes in mice from five consecutive generations (12). In our experiment no reproductive changes were observed. However, specific tests for ovary function or chromosome changes were not performed. *Mazhugan and co-workers* (13) reported disturbances of the metabolism of glycoaminoglycans in osteogenic cells in periostal and endostal bone following phenol intoxication in guinea pigs after two to three weeks of exposure. They assumed that the biosynthesis rates of sulphated glycoaminoglycans in the bone growth regions were reduced by phenol. Our animals had smaller bones but of the same density as controls which does not indicate a specific disturbance of the metabolism in osteogenic cells.

The fact that our animals exposed to the P effluent had lower body weights, atrophic organs and smaller bones compared with controls without changes in food consumption indicates disturbances in energy metabolism. Similar findings were not reported by other authors for phenol. The same applies to increased copper concentration in the carcasses — a finding not mentioned for phenols by other authors — whose singnificance is still unknown. Some of the observed changes in animals exposed to the P effluent may be due to the high pH (11) in the drinking solution. It should be mentioned that in the process of coal gasification the waste water from the Phenosolvan unit is supposed to undergo a process of biological treatment for degradation of phenol before being discharged into te river. Owing to this treatment a much lower concen-

tration of phenol is likely to occur in the environment of a coal gasification plant. The number of animals in our experiment and the experimental methods used might not have been sufficient for making firm conclusions especially in respect to possible carcinogenicity of these effluents.

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*Sažetak*

## REPRODUKCIJSKO-TOKSIKOLOŠKI UČINCI U ŠTAKORA NAKON ORALNE IZLOŽENOSTI OTPADNIM VODAMA IZ POSTROJENJA ZA UPLINJAVANJE UGLJENA

U ovom radu istražen je učinak triju otpadnih voda iz postupaka za uplinjavanje ugljena po Lurgiu na zdravlje štakora. Izložene skupine životinja dobivale su umjesto vode za piće Fleissnerov kondenzat (F), otpadnu vodu iz generatorske jedinice (G) ili fenosolvansku otpadnu vodu (P) tijekom triju generacija. Određivani reprodukcijski parametri bili su indeks plodnosti, tjelesne težine i broj mladunčadi u leglu. Učinci na zdravlje u štakora $F_1$, $F_2$ i $F_3$-generacije određivani su nakon 2 tjedna, odnosno 6 do 12 mjeseci trajanja postnatalne izloženosti. Pokazatelji učinaka bili su: tjelesne težine, potrošnja hrane i pića, koncentracije elemenata u tragovima u tijelu, hematološki parametri, morfometrijska mjerenja femura te obdukcijski i patohistološki nalazi.

Dobiveni rezultati su pokazivali da otpadne vode F i G ne izazivaju nikakve učinke na zdravlje štakora. U životinja izloženih fenosolvanskoj otpadnoj vodi (P) opaženo je sniženje tjelesnih težina, smanjena dnevna potrošnja pića, povišene koncentracije bakra u 14-dnevih štakora, smanjenje kostiju, hemosideroza jetre te atrofija bubrega i jetre.

Opažene promjene najvjerojatnije su posljedica štetnog učinka fenola koji se nalazi u fenosolvanskoj otpadnoj vodi.

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